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<b>UTILITY</b> <b>PATENT APPLICATION</b> <b>TRANSMITTAL</b> <small>(Only for new nonprovisional applications under 37 CFR 1.53(b))</small>		Attny Docket No.	<b>PZ038P1</b>	Total Pages:	<b>489</b>
		First Named Inventor or Application Identifier <b>Ruben et al.</b>			
APPLICATION ELEMENTS <small>See MPEP chapter 600 concerning utility patent application contents</small>		Date	<b>September 13, 2000</b>		
		Assistant Commissioner for Patents Address to: Box Patent Application Washington, D.C. 20231			
1. <input checked="" type="checkbox"/> Fee Transmittal Form (in duplicate) Total pgs: <b>1</b> 2. <input checked="" type="checkbox"/> Specification Total pages: <b>395</b> (preferred arrangement set forth below) - Descriptive title of the Invention - Cross References to Related Applications - Statement Regarding Fed Sponsored R&D - Reference to Microfiche Appendix - Background of the Invention - Brief Summary of the Invention - Brief Description of the Drawings (if filed) - Detailed Description - Claim(s) - Abstract of the Disclosure 3. a. Drawings Figures N/A Total sheets: b. Sequence Listing Total pages: <b>88</b> 4. <input checked="" type="checkbox"/> Unexecuted Declaration Total pages: <b>3</b> a. <input type="checkbox"/> Newly executed (original or copy) b. <input type="checkbox"/> Copy from a prior application (1.63(d)) (for con/div with Box 17 completed) i. <input type="checkbox"/> Deletion of Inventors (signed statement attached deleting inventor(s) named in prior app.) 5. <input checked="" type="checkbox"/> Incorporation by Reference (with Box 4b) The entire disclosure of the application referred to in Box 17 is hereby incorporated by reference.		6. <input type="checkbox"/> Microfiche Computer Program (appendix) 7. Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary) a. <input checked="" type="checkbox"/> Computer Readable Copy b. <input checked="" type="checkbox"/> Paper Copy (identical to computer copy) c. <input checked="" type="checkbox"/> Statement verifying identity of above copies			
		<b>Accompanying Application Parts</b> 8. <input type="checkbox"/> Assignment Papers (cover and document(s)) 9. <input type="checkbox"/> 37 CFR 3.73(b) Statement <input type="checkbox"/> Power of Attny 10. <input type="checkbox"/> English Translation Document 11. <input type="checkbox"/> Information Disclosure Statement/PTO-1449  <input type="checkbox"/> Copies of IDS Citations 12. <input type="checkbox"/> Preliminary Amendment 13. <input checked="" type="checkbox"/> Return Receipt Postcard (MPEP 503) (should be specifically itemized) 14. <input type="checkbox"/> Small Entity Statement(s) <input type="checkbox"/> Statement filed in prior application, status still proper and desired 15. <input type="checkbox"/> Certified copy of priority document(s) 16. <input type="checkbox"/> Other:			
17. If a CONTINUING APPLICATION, check appropriate box and supply the requisite information:  <input type="checkbox"/> Continuation <input checked="" type="checkbox"/> Continuation-in-Part (CIP) of prior application No.: PCT/US00/06783, filed March 16, 2000, which claims benefit under 35 U.S.C. § 119(e) based on U.S. Provisional Application No. 60/125,055, filed March 18, 1999.					
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Ruben et al.

Filed: Concurrently herewith

For: 27 Human Secreted Proteins

Attorney Docket No.: PZ038P1

PATENT APPLICATION FEE SHEET

Assistant Commissioner for Patents  
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Washington, D.C. 20231

Sir:

The filing fee required in connection with the subject application is being filed concurrently herewith has been calculated as follows:

TYPE	NO. FILE	LESS	EXTRA	EXTRA RATE	FEE
Total Claims	23	-20	3	\$18.00 each	\$ 54.00
Independent	4	-3	1	\$78.00 each	\$ 78.00
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Multiple Dependency Fee					N/A
Total Filing Fee					<b>\$822.00</b>

This application is a continuation-in-part of, and claims benefit under 35 U.S.C. § 120 of co-pending PCT international application Serial No. PCT/US00/06783, filed March 16, 2000, which claims benefit under 35 U.S.C. § 119(e) based on U.S. Provisional Application No. 60/125,055, filed March 18, 1999.

Please charge the required fee to Deposit Account No. 08-3425. In addition, the Commissioner is hereby authorized to charge payment for any additional filing fees required under 37 C.F.R. 1.16 or credit any overpayment to Deposit Account No. 08-3425. A duplicate of this paper is attached.

Respectfully submitted,

Dated: September 13, 2000

  
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Enclosure

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## 27 Human Secreted Proteins

This application is a continuation-in-part of, and claims benefit under 35 U.S.C. § 120 of copending PCT International Application Serial No. PCT/US00/06783, filed March 16, 2000, which is hereby incorporated by reference, which claims benefit under 35 U.S.C. § 119(e) based on U.S. Provisional Application No. 60/125,055, filed March 18, 1999, which is hereby incorporated by reference.

### *Field of the Invention*

This invention relates to newly identified polynucleotides, polypeptides encoded by these polynucleotides, antibodies that bind these polypeptides, uses of such polynucleotides, polypeptides, and antibodies, and their production.

### *Background of the Invention*

Unlike bacterium, which exist as a single compartment surrounded by a membrane, human cells and other eucaryotes are subdivided by membranes into many functionally distinct compartments. Each membrane-bounded compartment, or organelle, contains different proteins essential for the function of the organelle. The cell uses "sorting signals," which are amino acid motifs located within the protein, to target proteins to particular cellular organelles.

One type of sorting signal, called a signal sequence, a signal peptide, or a leader sequence, directs a class of proteins to an organelle called the endoplasmic reticulum (ER). The ER separates the membrane-bounded proteins from all other types of proteins. Once localized to the ER, both groups of proteins can be further directed to another organelle called the Golgi apparatus. Here, the Golgi distributes the proteins to vesicles, including secretory vesicles, the cell membrane, lysosomes, and the other organelles.

Proteins targeted to the ER by a signal sequence can be released into the extracellular space as a secreted protein. For example, vesicles containing secreted proteins can fuse with the cell membrane and release their contents into the extracellular space - a process called exocytosis. Exocytosis can occur constitutively or after receipt of a triggering signal. In the latter case, the proteins are stored in secretory vesicles (or secretory granules) until exocytosis is triggered. Similarly,



proteins residing on the cell membrane can also be secreted into the extracellular space by proteolytic cleavage of a "linker" holding the protein to the membrane.

Despite the great progress made in recent years, only a small number of genes encoding human secreted proteins have been identified. These secreted proteins  
 5 include the commercially valuable human insulin, interferon, Factor VIII, human growth hormone, tissue plasminogen activator, and erythropoietin. Thus, in light of the pervasive role of secreted proteins in human physiology, a need exists for identifying and characterizing novel human secreted proteins and the genes that encode them. This knowledge will allow one to detect, to treat, and to prevent  
 10 medical diseases, disorders, and/or conditions by using secreted proteins or the genes that encode them.

### ***Summary of the Invention***

The present invention relates to novel polynucleotides and the encoded  
 15 polypeptides. Moreover, the present invention relates to vectors, host cells, antibodies, and recombinant and synthetic methods for producing the polypeptides and polynucleotides. Also provided are diagnostic methods for detecting diseases, disorders, and/or conditions related to the polypeptides and polynucleotides, and therapeutic methods for treating such diseases, disorders, and/or conditions. The  
 20 invention further relates to screening methods for identifying binding partners of the polypeptides.

### ***Detailed Description***

#### **Definitions**

25 The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated  
 30 polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of



matter, or particular cell is not the original environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA  
 5 preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention.

In the present invention, a "secreted" protein refers to those proteins capable of being directed to the ER, secretory vesicles, or the extracellular space as a result of  
 10 a signal sequence, as well as those proteins released into the extracellular space without necessarily containing a signal sequence. If the secreted protein is released into the extracellular space, the secreted protein can undergo extracellular processing to produce a "mature" protein. Release into the extracellular space can occur by many mechanisms, including exocytosis and proteolytic cleavage.

In specific embodiments, the polynucleotides of the invention are at least 15,  
 15 at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000 continuous nucleotides but are less than or equal to 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb, 7.5 kb, 5 kb, 2.5 kb, 2.0 kb, or 1 kb, in length. In a further embodiment, polynucleotides of the invention comprise a portion of the coding sequences, as  
 20 disclosed herein, but do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or  
 25 1 genomic flanking gene(s).

As used herein, a "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:X or the cDNA contained within the clone deposited with the ATCC. For example, the polynucleotide can contain the nucleotide sequence of the full length cDNA sequence, including the 5' and 3'  
 30 untranslated sequences, the coding region, with or without the signal sequence, the secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a "polypeptide" refers to a



molecule having the translated amino acid sequence generated from the polynucleotide as broadly defined.

In the present invention, the full length sequence identified as SEQ ID NO:X was often generated by overlapping sequences contained in multiple clones (contig analysis). A representative clone containing all or most of the sequence for SEQ ID NO:X was deposited with the American Type Culture Collection ("ATCC"). As shown in Table 1, each clone is identified by a cDNA Clone ID (Identifier) and the ATCC Deposit Number. The ATCC is located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

A "polynucleotide" of the present invention also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:X, the complement thereof, or the cDNA within the clone deposited with the ATCC. "Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65 degree C.

Also contemplated are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH<sub>2</sub>PO<sub>4</sub>; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).



Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA<sup>+</sup> sequences (such as any 3' terminal polyA<sup>+</sup> tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).

The polynucleotide of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

The polypeptide of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well



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to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention.)

Many proteins (and translated DNA sequences) contain regions where the amino acid composition is highly biased toward a small subset of the available residues. For example, membrane spanning domains and signal peptides (which are also membrane spanning) typically contain long stretches where Leucine (L), Valine (V), Alanine (A), and Isoleucine (I) predominate. Poly-Adenosine tracts (polyA) at the end of cDNAs appear in forward translations as poly-Lysine (poly-K) and poly-Phenylalanine (poly-F) when the reverse complement is translated. These regions are often referred to as “low complexity” regions.

Such regions can cause database similarity search programs such as BLAST to find high-scoring sequence matches that do not imply true homology. The problem is exacerbated by the fact that most weight matrices (used to score the alignments generated by BLAST) give a match between any of a group of hydrophobic amino acids (L, V and I) that are commonly found in certain low complexity regions almost as high a score as for exact matches.

In order to compensate for this, BLASTX.2 (version 2.0a5MP-WashU) employs two filters (“seg” and “xnu”) which “mask” the low complexity regions in a particular sequence. These filters parse the sequence for such regions, and create a new sequence in which the amino acids in the low complexity region have been replaced with the character “X”. This is then used as the input sequence (sometimes referred to herein as “Query” and/or “Q”) to the BLASTX program. While this regime helps to ensure that high-scoring matches represent true homology, there is a negative consequence in that the BLASTX program uses the query sequence that has been masked by the filters to draw alignments.

Thus, a stretch of “X”s in an alignment shown in the following application does not necessarily indicate that either the underlying DNA sequence or the translated protein sequence is unknown or uncertain. Nor is the presence of such stretches meant to indicate that the sequence is identical or not identical to the



sequence disclosed in the alignment of the present invention. Such stretches may simply indicate that the BLASTX program masked amino acids in that region due to the detection of a low complexity region, as defined above. In all cases, the reference sequence(s) (sometimes referred to herein as “Subject”, “Sbjct”, and/or “S”) indicated  
 5 in the specification, sequence table (Table 1), and/or the deposited clone is (are) the definitive embodiment(s) of the present invention, and should not be construed as limiting the present invention to the partial sequence shown in an alignment, unless specifically noted otherwise herein.

## 10 **Polynucleotides and Polypeptides of the Invention**

### **FEATURES OF PROTEIN ENCODED BY GENE NO: 1**

The translation product of this gene shares sequence homology with mouse  
 15 semaphorin B (See Genbank Accession No.: emb|CAA59983.1|, all references available through this accession are hereby incorporated in their entirety herein), which is thought to be important in nerve cell growth and regeneration, axon guidance and nervous system development and function (see, e.g., Puschel et al., Neuron 14:941-948 (1995)). It is expected that the present invention will share certain  
 20 biological functions with the mouse semaphorin B protein which are known in the art. Furthermore, assays for this activity are well known and described in the art.

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences:  
 RPSWYXCRYRSGIPGSTHASG (SEQ ID NO: 91). Moreover, fragments and  
 25 variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind  
 30 polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.



The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 687-703 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 704-761 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type Ib membrane proteins.

This gene is expressed primarily in fetal and infant brain tissues, dendritic cells, testis and placental tissue, and to a lesser extent in pancreas tissue, kidney cortex tissue, B and T-cells and lymphomas.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, aberrant central nervous system development and function, neurodegenerative and immune conditions. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system and lymphoid systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cells sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 51 as residues: Gly-32 to Pro-38, Ala-105 to Ser-111, Lys-116 to Gln-123, Gly-172 to Ala-181, Leu-278 to Trp-283, Gly-352 to Arg-375. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in brain and immune cells and homology to semaphorins indicates that polynucleotides and polypeptides corresponding to this gene would be useful for the study and/or treatment of disorders of central nervous system development and maintenance, as well as brain, lymphoid and other



neoplasms. Likewise, neutralizing antibodies against this and other semaphorins, by inhibiting their axon-repelling action, are useful in treating neurodegenerative conditions.

Moreover, polynucleotides and polypeptides corresponding to this gene would be useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception.

In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function and development. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:11 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the



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Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample



and for diagnosis of diseases and conditions which include, but are not limited to, cancer and other proliferative disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the skin, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., skin, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cells sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 52 as residues: Met-1 to Ala-8, Pro-46 to His-54, Pro-56 to Leu-64, Ser-71 to Asp-76, Glu-83 to Trp-91, Leu-133 to Gln-141, Pro-152 to Asp-161, Thr-206 to Asp-214, Pro-225 to Gln-236, Lys-248 to Gly-258, Trp-273 to Gly-285. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution of this apparent splice variant of human neuropsin indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of cancer and other proliferative disorders. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:12 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1090 of SEQ ID NO:12, b is an integer of 15 to 1104, where both a and b correspond to the positions of



nucleotide residues shown in SEQ ID NO:12, and where b is greater than or equal to a + 14.

### FEATURES OF PROTEIN ENCODED BY GENE NO: 3

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The translation product of this gene shares sequence homology with a C. elegans protein (See, e.g., Genbank Accession No.: >gi|3980035).

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

- 10 QLDGVGLESRSPGCSTWEKADRVRG PVAQRAVASGSGKWRQEP SLHFAMSF  
LIDSSIMITSQILFFGFGWLFFMRQLFKDYEIRQYVVQVIFSVTFAFSCTMFELII  
FEILGVLNSSSRYPFWK (SEQ ID NO: 92),  
QLDGVGLESRSPGCSTWEKADRVRG PVAQRAVASGSGKWRQEP (SEQ ID  
NO: 93), SLHFAMSFLIDSSIMITSQILFFGFGWLFFMRQLFKDYEIRQYV (SEQ  
15 ID NO: 94), and/or VQVIFSVTFAFSCTMFELII FEILGVLNSSSRYPFWK (SEQ ID  
NO: 95). Moreover, fragments and variants of these polypeptides (such as, for  
example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%,  
96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by  
the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide  
20 encoding these polypeptides, or the complement thereof are encompassed by the  
invention. Antibodies that bind polypeptides of the invention are also encompassed by  
the invention. Polynucleotides encoding these polypeptides are also encompassed by  
the invention.

- 25 The gene encoding the disclosed cDNA is thought to reside on chromosome 1.  
Accordingly, polynucleotides related to this invention are useful as a marker in  
linkage analysis for chromosome 1.

This gene is expressed primarily in brain, fetal heart, lung carcinoma tissues.

- 30 Polynucleotides and polypeptides of the invention are useful as reagents for  
differential identification of the tissue(s) or cell type(s) present in a biological sample  
and for diagnosis of diseases and conditions which include, but are not limited to,  
brain cancer and lung carcinoma. Similarly, polypeptides and antibodies directed to  
these polypeptides are useful in providing immunological probes for differential



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nucleotide residues shown in SEQ ID NO:13, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 4**

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This gene is expressed primarily in early stage (9 weeks) human tissues.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, developmental, reproductive and metabolic defects, and cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the human embryo, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., embryonic, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cells sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 54 as residues: Asn-25 to Gln-39, Gly-82 to Arg-87, Arg-94 to Phe-101, Arg-134 to Arg-147. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in embryonic tissues indicates that polynucleotides and polypeptides corresponding to this gene would be useful for the detection, prevention, and/or treatment of morphogenetic and general developmental, reproductive and metabolic disorders and neoplasms. Furthermore, expression within embryonic tissue and other cellular sources marked by proliferating cells indicates that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus, this protein may also be involved in apoptosis or tissue



differentiation and could again be useful in cancer therapy. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:14 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 833 of SEQ ID NO:14, b is an integer of 15 to 847, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:14, and where b is greater than or equal to a + 14.

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## **FEATURES OF PROTEIN ENCODED BY GENE NO: 5**

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 516 - 532 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 533 - 552 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type Ia membrane proteins.

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences:  
PRVRPCRGESAGAAAAVPSQLPPRAAPPPARMLEEAGEVLEN (SEQ ID NO: 96). Moreover, fragments and variants of these polypeptides (such as, for example,



fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is believed to reside on chromosome 19. Accordingly, polynucleotides related to this invention would be useful as a marker in linkage analysis for chromosome 19.

This gene is expressed primarily in hematopoietic cells and epithelial cells, and to a lesser extent in several other tissues and cells including cancer cells, such as ovarian and breast cancer tissues.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, disorders of the hematopoietic and immune system and epithelial system. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the hematopoietic and immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, epithelial, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cells sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 55 as residues: Arg-39 to Arg-53, Gln-85 to Leu-90, Tyr-248 to Thr-253, Tyr-271 to Arg-276, Lys-315 to Thr-321, Ser-379 to Ser-386, Ser-393 to Thr-404, Thr-419 to Asp-426, Lys-



485 to Pro-491. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in haemopoietic and epithelial cells indicates that polynucleotides and polypeptides corresponding to this gene would be useful for the prevention, detection, treatment and/or diagnosis of disorders of the haemopoietic and epithelial system including cancers, especially breast cancer and ovarian cancer, as well as cancers of other tissues where expression has been indicated.

Furthermore, the tissue distribution in epithelial cells that polynucleotides and polypeptides corresponding to this gene would be useful for the treatment, diagnosis, and/or prevention of various skin disorders. Representative uses are described in the "Biological Activity", "Hyperproliferative Disorders", "Infectious Disease", and "Regeneration" sections below, in Example 11, 19, and 20, and elsewhere herein. Briefly, the protein is useful in detecting, treating, and/or preventing congenital disorders (i.e., nevi, moles, freckles, Mongolian spots, hemangiomas, port-wine syndrome), integumentary tumors (i.e., keratoses, Bowen's disease, basal cell carcinoma, squamous cell carcinoma, malignant melanoma, Paget's disease, mycosis fungoides, and Kaposi's sarcoma), injuries and inflammation of the skin (i.e., wounds, rashes, prickly heat disorder, psoriasis, dermatitis), atherosclerosis, urticaria, eczema, photosensitivity, autoimmune disorders (i.e., lupus erythematosus, vitiligo, dermatomyositis, morphea, scleroderma, pemphigoid, and pemphigus), keloids, striae, erythema, petechiae, purpura, and xanthelasma. In addition, such disorders may predispose increased susceptibility to viral and bacterial infections of the skin (i.e., cold sores, warts, chickenpox, molluscum contagiosum, herpes zoster, boils, cellulitis, erysipelas, impetigo, tinea, athlete's foot, and ringworm).

Moreover, polynucleotides and polypeptides corresponding to this gene may also be useful for the treatment or diagnosis of various connective tissue disorders (i.e., arthritis, trauma, tendonitis, chondromalacia and inflammation, etc.), autoimmune disorders (i.e., rheumatoid arthritis, lupus, scleroderma, dermatomyositis, etc.), dwarfism, spinal deformation, joint abnormalities, and chondrodysplasias (i.e., spondyloepiphyseal dysplasia congenita, familial osteoarthritis, Atelosteogenesis type II, metaphyseal chondrodysplasia type Schmid). Furthermore, the protein may also be used to determine biological activity, to raise



antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and immunotherapy targets for the above listed tumors and tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:15 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2161 of SEQ ID NO:15, b is an integer of 15 to 2175, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:15, and where b is greater than or equal to a + 14.

### FEATURES OF PROTEIN ENCODED BY GENE NO: 6

The translation product of this gene shares sequence homology with N-heparan sulfate sulfotransferase from rat (See Genbank Accession No.: pir|A42855), which catalyzes the transfer of sulfate from 3'-phosphoadenosine 5'-phosphosulfate to heparan sulfate. (Hashimoto, et al. (1992) JBC 5:267 pp15744-15750).

When tested against both Jurkat T-cells and U937 Myeloid cell lines, supernatants removed from cells containing this gene activated the GAS assay. Thus, it is likely that this gene activates both T-cells and myeloid cells, and to a lesser extent other immune cells, through the Jak-STAT signal transduction pathway. The gamma activating sequence (GAS) is a promoter element found upstream of many genes which are involved in the Jak-STAT pathway. The Jak-STAT pathway is a large, signal transduction pathway involved in the differentiation and proliferation of cells. Therefore, activation of the Jak-STAT pathway, reflected by the binding of the GAS element, can be used to indicate proteins involved in the proliferation and differentiation of cells.



In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences:

HKLLTEIGKVAGTPSFLLTIFYGASVGIVGESTYN (SEQ ID NO: 97). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention.

Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in Soares NhHMPu S1, PERM TF274, Hodgkin's Lymphoma II, Soares placenta Nb2HP, and Soares multiple sclerosis 2NbHMSP tissues, and to a lesser extent in a variety of normal and transformed adult and fetal tissues.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer and other proliferative disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cells sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 56 as residues: Ser-35 to Ser-44, Ser-86 to Leu-91, Asp-143 to Leu-150, Lys-166 to Ser-171, Ser-208



to Gly-213, Lys-239 to Leu-244, Glu-317 to Asn-324. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in immune tissues, and the homology to N-heparan sulfate sulfotransferase, indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of cancer and other proliferative disorders. Furthermore, expression within cellular sources marked by proliferating cells indicates that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Likewise, this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:16 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1341 of SEQ ID NO:16, b is an integer of 15 to 1355, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:16, and where b is greater than or equal to a + 14.

## **FEATURES OF PROTEIN ENCODED BY GENE NO: 7**

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences:

GRVEGPPAWEAAPWPSLPCGPCIPI (SEQ ID NO: 98). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to



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This gene is expressed primarily in colon tissue.

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The tissue distribution in colon tissue indicates that polynucleotides and polypeptides corresponding to this gene would be useful for the prevention, treatment



and/or diagnosis of disorders of the digestive and immune systems. Expression in the colon tissue indicates the gene or its products would be useful for the diagnosis, treatment and/or prevention of disorders of the colon, including inflammatory disorders such as, diverticular colon disease (DCD), inflammatory colonic disease, Crohn's disease (CD), non-inflammatory bowel disease (non-IBD) colonic inflammation; ulcerative disorders such as, ulcerative colitis (UC), amebic colitis, eosinophilic colitis; noncancerous tumors, such as, polyps in the colon, adenomas, leiomyomas, lipomas, and angiomas. Furthermore, the tissue distribution in gastrointestinal tissue (colon tissue) indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis, prevention, and/or treatment of various metabolic disorders such as Tay-Sachs disease, phenylketonuria, galactosemia, porphyrias, and Hurler's syndrome. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:17 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2164 of SEQ ID NO:17, b is an integer of 15 to 2178, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:17, and where b is greater than or equal to a + 14.

## 30 FEATURES OF PROTEIN ENCODED BY GENE NO: 8



In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

- NLWGLQPRPPASLLQPTASYSRKDKDQRKQQAMWRVPSDLKMLKRLKTQM  
AEVRCMKTDVKNTLSEIKSSSAASGDMQTSLSADQAALAACGTENSGRLQD  
5 LGMELLAKSSVANCYIRNSTNKKNSPKPARSSVAGSLSLRRAVDPGENSRSK  
GDCQTLSEGSPGSSQSGSRHSSPRALIHGSIGDILPKTEDRQCKALDSDAVVVA  
VFSGLPAVEKRRKMVTLGANAKGGHLEGLQMTDLENNSETGELQPVLPESA  
SAAPEEGMSSSDSIECDTENEEQEEHTSVGGFHDSFMVMTQPPDEDTHSSFPD  
GEQIGPEDLSFNTDENSGR (SEQ ID NO: 99),  
10 NLWGLQPRPPASLLQPTASYSRKDKDQRKQQAMWRVPSDL (SEQ ID NO:  
100), KMLKRLKTQMAEVRCMKTDVKNTLSEIKSSSAASGDMQTSLS (SEQ ID  
NO: 101), FSADQAALAACGTENSGRLQDLGMELLAKSSVANCYIRNST (SEQ  
ID NO: 102), NKKNSPKPARSSVAGSLSLRRAVDPGENSRSKGDCQTLSEG  
(SEQ ID NO: 103),  
15 SPGSSQSGSRHSSPRALIHGSIGDILPKTEDRQCKALDSDAVVV (SEQ ID NO:  
104), AVFSGLPAVEKRRKMVTLGANAKGGHLEGLQMTDLENNSETG (SEQ  
ID NO: 105), ELQPVLPESASAAPEEGMSSSDSIECDTENEEQEEHTSVGGFHD  
(SEQ ID NO: 106), and/or  
SFMVMTQPPDEDTHSSFPDGEQIGPEDLSFNTDENSGR (SEQ ID NO: 107).  
20 Moreover, fragments and variants of these polypeptides (such as, for example,  
fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%,  
97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the  
polynucleotide which hybridizes, under stringent conditions, to the polynucleotide  
encoding these polypeptides, or the complement thereof are encompassed by the  
25 invention. Antibodies that bind polypeptides of the invention are also encompassed by  
the invention. Polynucleotides encoding these polypeptides are also encompassed by  
the invention.

This gene is expressed primarily in infant brain and placental tissues, and to a  
lesser extent in a number of other tissues, predominantly endocrine, hematopoietic  
30 and central nervous system structures.

Polynucleotides and polypeptides of the invention are useful as reagents for  
differential identification of the tissue(s) or cell type(s) present in a biological sample



and for diagnosis of diseases and conditions which include, but are not limited to, placental, neurological, neurodevelopmental and hormonal defects. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For

5 a number of disorders of the above tissues or cells, particularly of the nervous, urogenital and endocrine systems, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., neural, endocrine, placental, developmental, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or

10 cells sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 58 as residues:

15 Arg-36 to Pro-43. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in infant brain and placental tissues indicates that polynucleotides and polypeptides corresponding to this gene would be useful for the detection, prevention and/or treatment of abnormalities related to nervous system

20 development and function, as well as reproductive, hormonal and neoplastic disorders. Furthermore, the tissue distribution in placental tissue indicates that polynucleotides and polypeptides corresponding to this gene would be useful for the diagnosis and/or treatment of disorders of the placenta. Specific expression within the placenta indicates that this gene product may play a role in the proper establishment

25 and maintenance of placental function. Alternately, this gene product may be produced by the placenta and then transported to the embryo, where it may play a crucial role in the development and/or survival of the developing embryo or fetus. Expression of this gene product in a vascular-rich tissue such as the placenta also indicates that this gene product may be produced more generally in endothelial cells

30 or within the circulation. In such instances, it may play more generalized roles in vascular function, such as in angiogenesis. It may also be produced in the vasculature and have effects on other cells within the circulation, such as hematopoietic cells. It



may serve to promote the proliferation, survival, activation, and/or differentiation of hematopoietic cells, as well as other cells throughout the body. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

5 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:18 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence  
10 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2215 of SEQ ID NO:18, b is an integer of 15 to 2229, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:18, and where b is greater than or equal to a  
15 + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 9**

In specific embodiments, polypeptides of the invention comprise, or  
20 alternatively consist of, the following amino acid sequences:  
HASGWACLGRRRRCRGFSFRPLHGGGCLTGSPSG (SEQ ID NO: 108). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide  
25 which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

30 In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise, or



HASGWACLGRRRCRGFSFRPLHGGGCLTGSPSGMRLTRKRLCSFLIALYCLFS  
LYAAHYHVFFGRRRQAPAGSPRGLRKGAAPARERRGREQSTLESEEWNPWEG  
DEKNEOOHRFKTSLQILDKSTKGKTDLSVQIWGKAAIGLYLWEHIFEGLLDPS

5 DVTAQWREGKSIVGRTQYSFITGPAVIPGYFSVDVNNVVLILNGREKAKIFYA  
TQWLLYAQNLVQIQKLQHLAVVLLGNEHCDNEWINPFLKRNGGFVELLFIIY  
DSPWINDVDVFQWPLGVATYRNFVVEASWSMLHDERPYLCNFLGTIYENSS  
RQALMNILKKDGNKLCWVSAREHWQPQETNESLKNYQDALLQSDLTLCPV  
GVNTECYRIYEACSYGSIPVVEDVMTAGNCGNTSVHHGAPLQLLKSMGAPFI  
10 FIKNWKELPAVLEKEKTIILQEKIERRKMLLQWYQHFKTELKMKFTNILESSFL  
MNNKS (SEQ ID NO: 109). Moreover, fragments and variants of these polypeptides  
(such as, for example, fragments as described herein, polypeptides at least 80%, 85%,  
90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides  
encoded by the polynucleotide which hybridizes, under stringent conditions, to the  
15 polynucleotide encoding these polypeptides , or the complement there of are  
encompassed by the invention. Antibodies that bind polypeptides of the invention are  
also encompassed by the invention. Polynucleotides encoding these polypeptides are  
also encompassed by the invention.

This gene is expressed primarily in merkel cells, and to a lesser extent in  
20 endothelial cells, heart tissue, and testes tissue.

Polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, dermatitis and eczema, and vascular disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the skin and vascular tissue, expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., skin, vascular, cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cells sample taken from an individual having such a disorder, relative to the standard



gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 59 as residues:

- 5 Arg-2 to Leu-8, Gly-30 to Gly-38, Ala-50 to Thr-83, Lys-90 to Thr-96, Glu-204 to Trp-210, Phe-214 to Gly-219, His-263 to Tyr-268, Tyr-277 to Arg-282, Lys-290 to Lys-296, Gln-307 to Gln-319. Polynucleotides encoding said polypeptides are also encompassed by the invention.

- 10 The tissue distribution in merkel cells indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment and/or detection of defects in reduced sensitivity in the digital pads, lips and oral cavity, or in other dermatological diseases and conditions such as eczema or psoriasis. Furthermore, the tissue distribution in merkel cells indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment, diagnosis, and/or prevention
- 15 of various skin disorders including congenital disorders (i.e. nevi, moles, freckles, Mongolian spots, hemangiomas, port-wine syndrome), integumentary tumors (i.e. keratoses, Bowen's disease, basal cell carcinoma, squamous cell carcinoma, malignant melanoma, Paget's disease, mycosis fungoides, and Kaposi's sarcoma), injuries and inflammation of the skin (i.e. wounds, rashes, prickly heat disorder,
- 20 psoriasis, dermatitis), atherosclerosis, urticaria, eczema, photosensitivity, autoimmune disorders (i.e. lupus erythematosus, vitiligo, dermatomyositis, morphea, scleroderma, pemphigoid, and pemphigus), keloids, striae, erythema, petechiae, purpura, and xanthelasma. Moreover, such disorders may predispose increased susceptibility to viral and bacterial infections of the skin (i.e. cold sores, warts, chickenpox,
- 25 molluscum contagiosum, herpes zoster, boils, cellulitis, erysipelas, impetigo, tinea, athletes foot, and ringworm). Likewise, the tissue distribution in endothelial cells and heart tissue indicates that the protein product of this gene is useful for the diagnosis and treatment of conditions and pathologies of the cardiovascular system, such as heart disease, restenosis, atherosclerosis, stroke, angina, thrombosis, and wound
- 30 healing. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.



Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:19 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1500 of SEQ ID NO:19, b is an integer of 15 to 1514, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:19, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 10

The translation product of this gene shares sequence homology with seven transmembrane G-protein coupled receptors, including the leukotriene b4 receptor and CMKRL1, a chemoattractant receptor (see, e.g. Genbank Accession Nos. gi|1613771|gb|AAB16747.1| and gi|1648870|emb|CAA67001.1| all references available through these accessions are hereby incorporated in their entirety herein), which are thought to be important in external signal reception and transduction.

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 37 - 54 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 54 - 211 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type Ib membrane proteins.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:  
 PGNGFVWVSLAGWRPARGRPLAATLVHLALADGAVLLLTPLFVAFLTRQA  
 WPLGQAGCKAVYYVCAL (SEQ ID NO: 110),  
 FGLLWAPYHAVNLLQAVAALAPPEGALAKLGGAGQAARAGTTALAFFSSSV  
 NPVLYVFTAGDLLPRAGPRFLTRLFEGSGEARGG (SEQ ID NO: 111),



YRHLWRDRVQCQLCHPSPVHAAHLSLETLTAFVLPFGLMLGCYSVTLARLR  
GARWGSGRHGARVGRL VSAIV (SEQ ID NO: 112),

APRLLLLNLSASPGPQSCSLHPAWERDTAELEDFAGHRHSLPAAGGAAGAAW  
QRLRGVELGGLAACTGATAGGHACAAPGAGRRRGAAAHAALCGLPDPASL

5 AAGPGGLQGGVLRVRAQHVRQRAAHRPAQPAALPRGHPPLPGASVRSPALA  
RRLLLAVWLAALLLAVPAAV (SEQ ID NO: 113),

PSSACSGPPTTQSTFCRRSQRWLHRKGPWRSWAEPARRRERELRPWPSSVLA  
STRCSTSSPLEICCPGQVPVSSRGSSKALGRPEGAAA (SEQ ID NO: 114), and

PGKPGRWARRAARRCTTCARSACTPACCSPACSAASRSPAPSWRLGAQP

10 GPGPPPAAGGLAGRPVARRPGRRLPPPVEGPRMPAVPPVAGPRRRPPEPGDSD  
RFRASFRADARLLQRDAGTAAGRPLGLRAARGAGGPAGERHRAF (SEQ ID

NO: 115). Moreover, fragments and variants of these polypeptides (such as, for  
example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%,  
96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by

15 the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide  
encoding these polypeptides , or the complement there of are encompassed by the  
invention. Antibodies that bind polypeptides of the invention are also encompassed by  
the invention. Polynucleotides encoding these polypeptides are also encompassed by  
the invention.

20 An additional preferred polypeptide fragment of the invention comprises the  
following amino acid sequence:

MYASVLLTGLLSLQRCLAVTRPFLAPRCAARPWPAACCWRSGWPPCCSPSRP  
PSTATCGGTAYASCATRRRSTPPPT (SEQ ID NO: 116). Moreover, fragments and

25 polypeptides (such as, for example, fragments as described herein,  
polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to  
these polypeptides and polypeptides encoded by the polynucleotide which hybridizes,  
under stringent conditions, to the polynucleotide encoding these polypeptides , or the  
complement there of are encompassed by the invention. Antibodies that bind  
polypeptides of the invention are also encompassed by the invention. Polynucleotides  
30 encoding these polypeptides are also encompassed by the invention.

This gene is expressed primarily in retinal tissue, and to a lesser extent in  
testis tumor, groin wound tissues, and eosinophils.



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Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:20 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1007 of SEQ ID NO:20, b is an integer of 15 to 1021, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:20, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 11**

The translation product of this gene shares sequence homology with a bovine ubiquitin-like protein, which is thought to be important in apoptosis.

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences:

VSPQKAASLVIRIRWRHVRPSPPSASRLRRLPPRHLTVAXRPRREGVGTGSRAV  
 LCILATCGSKMSDIGDWFRSIPAITRYWFAATVAVPLVGKLGLISPAYLFLWP  
 EAFLYRFQIWRPITATFYFPVGP GTGFLYLVNLYFLYQYSTRLETGAFDGRPA  
 DYLF (SEQ ID NO: 117),  
 VSPQKAASLVIRIRWRHVRPSPPSASRLRRLPPRHLTVAXRPRR (SEQ ID NO:  
 118), EGVGTGSRAVLCILATCGSKMSDIGDWFRSIPAITRYWFAATVA (SEQ  
 ID NO: 119), VPLVGKLGLISPAYLFLWP EAFLYRFQIWRPITATFYFPVGP GTG  
 (SEQ ID NO: 120), and/or FLYLVNLYFLYQYSTRLETGAFDGRP ADYLF (SEQ  
 ID NO: 121). Moreover, fragments and variants of these polypeptides (such as, for  
 example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%,  
 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by  
 the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide  
 encoding these polypeptides, or the complement thereof are encompassed by the  
 invention. Antibodies that bind polypeptides of the invention are also encompassed by



the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequence:

VSPQKAASLVIRWRHVRSPSPASRLRRLPPRHLTVAXRPRREGVGTGSRAV  
LCILATCGSKMSDIGDWFRSIPAITRYWFAATVAVPLVGKLGLISPAYLFLWP  
EAFLYRFQIWRPITATFYFPVGP GTGFLYLVNLYFLYQYSTRLETGAFDGRPA  
DYL FMLLFNWICIVITGLAMDMQLLMIPLMSVLYVWAQLNRDMIVSFWFGT  
RFKACYLPWVILGFNYIIGGSVINELIGNLVGHLYFFLMFRYPMDLGGRNFLS  
TPQFLYRWLPSRRGGVSGFGVPPASMRRAADQNGGXGRHNWGQGFR LGDQ  
(SEQ ID NO: 122). Moreover, fragments and variants of these polypeptides (such as,

for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is thought to reside on chromosome 8. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 8.

It has been discovered that this gene is expressed primarily in primary dendritic cells, T-cell lymphoma, and chronic lymphocytic leukemia.

Nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: T-cell lymphoma and chronic lymphocytic leukemia. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or



lower levels may be detected in certain tissues (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 61 as residues: Pro-111 to Gly-116, Ala-130 to Gly-136. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in primary dendritic cells, T-cell lymphoma, and chronic lymphocytic leukemia tissues, and the homology to apoptosis related genes, suggests that polynucleotides and polypeptides corresponding to this gene are useful for the detection and/or treatment of T-cell lymphomas and chronic lymphocytic leukemias, as well as cancers of other tissues where expression has been observed. Furthermore, expression within cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Likewise, this protein may also be involved in apoptosis or tissue differentiation, and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:21 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1845 of SEQ ID NO:21, b is an integer of 15 to 1859, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:21, and where b is greater than or equal to a + 14.







lymphoid origin, indicates the natural gene product would be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:22 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1480 of SEQ ID NO:22, b is an integer of 15 to 1494, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:22, and where b is greater than or equal to a + 14.

### FEATURES OF PROTEIN ENCODED BY GENE NO: 13



The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 3 - 19 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 20 - 322 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type Ib membrane proteins.

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

- 10 AARGLYDYGSGLCWAWAARPSSFVSGSSREAPSATAAPSWTRSVTAASAAA  
ASRMAMCSSTRPARLLLPPPTTPSPRPRTLTPVDPCSGGCRLTSKDHTPRVGT  
GQGRGQGTFWLSRDEGYFAEDTRIGHFQDSLPAPLPLPSFEALIKHKSGSPGA  
VCQRWAGGETDRGCG (SEQ ID NO: 123),  
AARGLYDYGSGLCWAWAARPSSFVSGSSREAPSATAAPS (SEQ ID NO: 124),  
15 WTRSVTAASAAAASRMAMCSSTRPARLLLPPPTTPSPRP (SEQ ID NO: 125),  
RTLTPVDPCSGGCRLTSKDHTPRVGTGQGRGQGTFWLSRDE (SEQ ID NO:  
126), GYFAEDTRIGHFQDSLPAPLPLPSFEALIKHKSGSPGAVCQR (SEQ ID  
NO: 127), and/or WAGGETDRGCG (SEQ ID NO: 128). Moreover, fragments and  
variants of these polypeptides (such as, for example, fragments as described herein,  
20 polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to  
these polypeptides and polypeptides encoded by the polynucleotide which hybridizes,  
under stringent conditions, to the polynucleotide encoding these polypeptides , or the  
complement there of are encompassed by the invention. Antibodies that bind  
polypeptides of the invention are also encompassed by the invention. Polynucleotides  
25 encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is thought to reside on chromosome 11. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 11.

- 30 It has been discovered that this gene is expressed primarily in kidney cortex  
and fetal and infant brain tissues, and to a lesser extent in  
Soares\_fetal\_heart\_NbHH19W tissue.



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sexually-linked disorders. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly  
 5 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:23 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention  
 10 are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2091 of SEQ ID NO:23, b is an integer of 15 to 2105, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:23, and where b is greater than or equal to a + 14.

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#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 14**

It has been discovered that this gene is expressed primarily in pancreatic islet cells, T-cells and dendritic cells.

20 Nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: diabetes, autoimmune disorders, and immunodeficiencies. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification  
 25 of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be detected in certain tissues (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene  
 30 expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.



The tissue distribution in immune tissues suggests that polynucleotides and polypeptides corresponding to this gene are useful for the treatment and/or detection of disease disorders associated with the pancreas including diabetes, in addition to immune disorders including autoimmune diseases, immunodeficiencies, arthritis and asthma. More generally, the tissue distribution in pancreas tissue suggests that polynucleotides and polypeptides corresponding to this gene are useful for the detection, treatment, and/or prevention of various endocrine disorders and cancers, particularly Addison's disease, Cushing's Syndrome, and disorders and/or cancers of the pancreas (e.g. diabetes mellitus), adrenal cortex, ovaries, pituitary (e.g., hyper-, hypopituitarism), thyroid (e.g. hyper-, hypothyroidism), parathyroid (e.g. hyper-, hypoparathyroidism), hypothalamus, and testes. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:24 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1276 of SEQ ID NO:24, b is an integer of 15 to 1290, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:24, and where b is greater than or equal to a + 14.

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#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 15**

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences:

APVSIIPFCVCPCVQNVLLPL (SEQ ID NO: 129). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to



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perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function, such as neuronal survival; synapse formation; conductance; neural differentiation, etc. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:25 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1714 of SEQ ID NO:25, b is an integer of 15 to 1728, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:25, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 16**

It has been discovered that this gene is expressed primarily in Helper T cells. Nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: immune system disorders and inflammatory disorders. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune, expression of this gene at significantly higher or lower



levels may be detected in certain tissues (e.g., immune, inflamed, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution of this gene predominantly in immune cell types suggests that the gene could be important for the treatment and/or detection of immune or hematopoietic disorders including arthritis, asthma, inflammatory disorders, and immunodeficiency diseases. Furthermore, expression of this gene product in T-cells suggests a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product may be involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses). Since the gene is expressed in cells of lymphoid origin, the gene or protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:26 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1555 of SEQ ID NO:26, b



is an integer of 15 to 1569, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:26, and where b is greater than or equal to a + 14.

## 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 17

In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences:

MFLLDGSNWILHCPITLRTYTTNLSIKFSKCSVNIYSLENKXFFSKKKKKKKRKE

- 10 NNPNGNKISNGEISVTLTGICKIFWKRAPFFFHFQSYLWCSYRVQTSRSF (SEQ ID NO: 130). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide
- 15 encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

- 20 It has been discovered that this gene is expressed primarily in neutrophils, stimulated with IL-1 and LPS.

- 25 Nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: inflammation; arthritis; hematopoietic disorders; susceptibility to infection; immune system dysfunction; autoimmune disorders. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels may be detected in certain tissues (e.g., immune, cancerous and wounded
- 30 tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene



expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution in neutrophils suggests that that polynucleotides and polypeptides corresponding to this gene would be useful for the diagnosis and/or treatment of various immune system disorders. Elevated expression of this gene product by activated neutrophils suggests that it may be involved in some of the processes performed by these cells, including inflammation, extravasation, tissue destruction, and recruitment of other cell types. Some such functions may implicate this gene product in a negative role, where treatments designed to block the activity of this factor could prove beneficial. Roles in tissue destruction and extravasation may also have implications in the treatment of cancer and neoplastic metastases. Alternately, expression by neutrophils may be reflective of more general functions in hematopoiesis, including effects on cell proliferation, survival, differentiation, and/or activation. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:27 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1044 of SEQ ID NO:27, b is an integer of 15 to 1058, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:27, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 18**

The translation product of this gene shares sequence homology with a C. elegans acid-rich protein (see Genbank Accession No.: gi|156195).



GRGPTAPAVRDPNAIPAQRSMAATDSMRGEAPGAETPSLRHRGQAAQPEPST  
GFTATPPAPDSPQEPLVLRLLKFLNDSEQVARAWPHDTIGSLKRTQFPGREQQV  
5 RLIYQGQLLGDDTQTLGSLHLPNCVLHCHVSTRVGPPNPPCPPGSEPGPSGL  
EIGSLLLPLLLLLLLLLLWYCQIQYRPFPLTATLGLAGFTLLLSLLAFAMYRP  
(SEQ ID NO: 131),  
GRGPTAPAVRDPNAIPAQRSMAATDSMRGEAPGAETPSLRHR (SEQ ID NO:  
132), GQAAQPEPSTGFTATPPAPDSPQEPLVLRLLKFLNDSEQVARAW (SEQ ID  
10 NO: 133), PHDTIGSLKRTQFPGREQQVRLIYQGQLLGDDTQTLGSLHLPNCV  
(SEQ ID NO: 134),  
LHCHVSTRVGPPNPPCPPGSEPGPSGLEIGSLLLPLLLLLLLLLLWY (SEQ ID  
NO: 135), and/or CQIQYRPFPLTATLGLAGFTLLLSLLAFAMYRP (SEQ ID NO:  
136). Moreover, fragments and variants of these polypeptides (such as, for example,  
15 fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%,  
97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the  
polynucleotide which hybridizes, under stringent conditions, to the polynucleotide  
encoding these polypeptides, or the complement thereof are encompassed by the  
invention. Antibodies that bind polypeptides of the invention are also encompassed by  
20 the invention. Polynucleotides encoding these polypeptides are also encompassed by  
the invention.

TRPGIWGQAARGAWRDFQRRRGLGSAAGKAGAMTLIEGVGDEVTVLFSVLA  
CLLVLALAWVSTHTAEGGDPLPQPSGTPTPSQPSAAMAATDSMRGEAPGAET  
PSLRHRGQAAQPEPSTGFTATPPAPDSPQEPLVLRLKFLNDSEQVARAWPHDT  
IGSLKRTQFPGREQQVRLIYQGQLLGDDTQTLGSLHLPPNCVLHCHVSTRVGP  
30 PNPPCPPGSEPRPLRAGNRQPAAAPAAPAVAAALVLPDPVPALLSPDRHSGPG  
RLHPAPQSPGLCHVPPVPPRALGSGVAGPSGPCSPRRGGSCCLPRPASACLFP  
LPWSPALRRRGLPGLAEAPPCDRRGSGPPPGAADPQPALGVGSSSGSGICCRCL



GPGQSRAAPGARLSVLPEDPAASNP (SEQ ID NO: 137). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

It has been discovered that this gene is expressed primarily in germinal center B cells, and to a lesser extent in various different tumor cells, such as colon tumor tissue.

Nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: immune system disorders and cancers of the colon, ovary, breast, and prostate. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and cancer, expression of this gene at significantly higher or lower levels may be detected in certain tissues (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 68 as residues: Glu-35 to Pro-52, Asp-60 to Glu-65, Arg-75 to Thr-87, Pro-94 to Gln-100, Lys-129 to Gln-139, Val-175 to Pro-198, Pro-223 to Arg-231, Pro-262 to Gly-269, Pro-289 to Gly-295, Pro-303 to Pro-313. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution of this gene predominantly in cancerous tissues suggests that the gene could be important for the treatment and/or detection of tumors



in a wide variety of tissues including colon/intestine, breast, ovary and prostate, as well as cancers of other tissues where expression has been observed. Expression within cellular sources marked by proliferating cells suggests that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Likewise, this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:28 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1339 of SEQ ID NO:28, b is an integer of 15 to 1353, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:28, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 19**

The gene encoding the disclosed cDNA is thought to reside on chromosome 14. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 14.

It has been discovered that this gene is expressed primarily in breast cancer tissue, and to a lesser extent in ovarian tumor tissue and most other tissue types.

Nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: breast and ovarian cancer. Similarly, polypeptides and antibodies directed to those polypeptides are useful to



provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the female reproductive system, expression of this gene at significantly higher or lower levels may be detected in certain tissues (e.g., reproductive, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution in breast and ovarian tumor tissues suggests that polynucleotides and polypeptides corresponding to this gene are useful for the treatment and/or detection of disorders associated with the female reproductive system, including ovarian and breast cancer. Furthermore, the translation product of this gene may be useful for the detection and/or treatment of cancers of other tissues where expression of this gene has been observed. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:29 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1064 of SEQ ID NO:29, b is an integer of 15 to 1078, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:29, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 20**



The gene encoding the disclosed cDNA is thought to reside on chromosome 2. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 2.

- In specific embodiments, polypeptides of the invention comprise, or  
 5 alternatively consists of, an amino acid sequence selected from the group:  
 MDRRFKLWEVFGKEKCEFKGSLSGSNAGITSIEFDSAGSYLLAASNDFASRIWT  
 VDDYRLRHTLTGHSGKVLSAKFLLDNARIVSGSHDRTLKLWDLRSKVCIKTV  
 FAGSSCNDIVCTEQCVMSGHFDKKIRFWDIRSEIVREMELLGKITALDLNPER  
 TELLSCSRDDLLKVIDLRTNAIKQTFSAPGFKCGSDWTRVVFSPDGSYVAAGS  
 10 AEGSLYIWSVLTGKVEKVLSKQHSSSINAVAWSPSGSHVVSVDKGCKAVLW  
 AQY (SEQ ID NO: 138),  
 MDRRFKLWEVFGKEKCEFKGSLSGSNAGITSIEFDSAGSYLLAASNDFASRIWT  
 (SEQ ID NO: 139),  
 VDDYRLRHTLTGHSGKVLSAKFLLDNARIVSGSHDRTLKLWDLRSKVCIKTV  
 15 F (SEQ ID NO: 140),  
 AGSSCNDIVCTEQCVMSGHFDKKIRFWDIRSEIVREMELLGKITALDLNPER  
 (SEQ ID NO: 141),  
 TELLSCSRDDLLKVIDLRTNAIKQTFSAPGFKCGSDWTRVVFSPDGSYVAAGS  
 (SEQ ID NO: 142), and/or  
 20 AEGSLYIWSVLTGKVEKVLSKQHSSSINAVAWSPSGSHVVSVDKGCKAVLW  
 AQY (SEQ ID NO: 143). Moreover, fragments and variants of these polypeptides  
 (such as, for example, fragments as described herein, polypeptides at least 80%, 85%,  
 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides  
 encoded by the polynucleotide which hybridizes, under stringent conditions, to the  
 25 polynucleotide encoding these polypeptides, or the complement thereof are  
 encompassed by the invention. Antibodies that bind polypeptides of the invention are  
 also encompassed by the invention. Polynucleotides encoding these polypeptides are  
 also encompassed by the invention.

- It has been discovered that this gene is expressed primarily in fetal liver,  
 30 uterus, brain and heart tissues, and to a lesser extent in various other tissues,  
 predominantly endocrine organs.



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T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and immunotherapy targets for the above listed tumors and tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:30 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2398 of SEQ ID NO:30, b is an integer of 15 to 2412, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:30, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 21**

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Included in this invention as preferred domains are Cysteine protease inhibitor signature domains, which were identified using the ProSite analysis tool (Swiss



Institute of Bioinformatics). Structurally, members of the family of cysteine protease inhibitors consist of a conserved region of five residues which appear to be important for binding to the cysteine proteases.

A signature pattern beginning one residue before this conserved region was  
 5 chosen as the consensus pattern as follows: [GSTEQKRV]-Q-[LIVT]-[VAF]-  
 [SAGQ]-G-x-[LIVMNK]-x(2)- [LIVMFY]-x-[LIVMFYA]-[DENQKRHSIV].  
 Preferred polypeptides of the invention comprise the following amino acid sequence:  
 SQLASGKLSKYWAI (SEQ ID NO: 144). Polynucleotides encoding these  
 polypeptides are also encompassed by the invention.

10 Further preferred are polypeptides comprising the cysteine proteases inhibitors  
 signature domains of this gene, and at least 5, 10, 15, 20, 25, 30, 50, or 75 additional  
 contiguous amino acid residues of the amino acid sequence referenced in Table 1 for  
 this gene. The additional contiguous amino acid residues may be N-terminal or C-  
 terminal to the cysteine proteases inhibitors signature domains. Alternatively, the  
 15 additional contiguous amino acid residues may be both N-terminal and C-terminal to  
 the cysteine proteases inhibitors signature domains, wherein the total N- and C-  
 terminal contiguous amino acid residues equal the specified number. The above  
 preferred polypeptide domain is characteristic of a signature specific to cysteine  
 proteases inhibitors proteins.

20 It has been discovered that this gene is expressed primarily in macrophage and  
 dendritic cells.

Nucleic acids of the invention are useful as reagents for differential  
 identification of the tissue(s) or cell type(s) present in a biological sample and for  
 diagnosis of the following diseases and conditions: immunity related disorders or  
 25 conditions. Similarly, polypeptides and antibodies directed to those polypeptides are  
 useful to provide immunological probes for differential identification of the tissue(s)  
 or cell type(s). For a number of disorders of the above tissues or cells, particularly of  
 the immune system, expression of this gene at significantly higher or lower levels  
 may be detected in certain tissues (e.g., immune, cancerous and wounded tissues) or  
 30 bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid or spinal fluid) taken  
 from an individual having such a disorder, relative to the standard gene expression



level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution in primary dendritic cells and macrophage indicates that polynucleotides and polypeptides corresponding to this gene would be useful for the diagnosis, prevention, prognosis and/or treatment of immunity related diseases or conditions, such as phagocytic defense against microorganisms, antigen pinocytosis, processing, and the presentation to B- and T-lymphocytes, the regulation of the production of interleukin or cytokines, the modulation of inflammatory response(s), the killing of tumor cells, and the regulation of hematopoiesis and lymphopoiesis, for example. Other representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. The cysteine protease inhibitor activity and/or an involvement in the regulation of cytokine production, antigen presentation, or other processes suggests a usefulness in the detection and/or treatment of cancer (e.g., by boosting immune responses). Expression in cells of lymphoid origin, indicates the natural gene product would be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, expression of this gene product in macrophage and primary dendritic cells also strongly suggests a role for this protein in immune function and immune surveillance. Protein, as well as, antibodies directed against the



protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:31 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1722 of SEQ ID NO:31, b is an integer of 15 to 1736, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:31, and where b is greater than or equal to a + 14.

## 15 **FEATURES OF PROTEIN ENCODED BY GENE NO: 22**

It has been discovered that this gene is expressed primarily in pulmonary tissue, leukemia and lymphoma cell lines and fetal tissue.

Nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: immunodeficiency, tumor necrosis, infection, lymphomas, auto-immunities, cancer, metastasis, inflammation, anemias (leukemia) and other hematopoietic disorders, as well as cardiovascular or respiratory/pulmonary disorders or infections. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the pulmonary and immune systems, expression of this gene at significantly higher or lower levels may be detected in certain tissues (e.g., immune, pulmonary, fetal, cancerous and wounded tissues) or bodily fluids (e.g., lymph, sputum, serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene



expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

The tissue distribution in pulmonary and immune tissues indicates that polynucleotides and polypeptides corresponding to this gene would be useful for the diagnosis, prognosis, prevention and/or treatment of variety of immune system disorders. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. Involvement in the regulation of cytokine production, antigen presentation, or other processes suggests a usefulness in the treatment of cancer (e.g., by boosting immune responses). Expression in cells of lymphoid origin, indicates the natural gene product would be involved in immune functions. Therefore it may be also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, scleroderma and tissues. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. In addition, this gene product may be applicable in conditions of general microbial infection, inflammation or cancer. In addition, the expression of this gene product in pulmonary tissue suggests a possible role in the detection and treatment of cardiovascular and respiratory/pulmonary disorders or infections, including: asthma, pulmonary edema, pneumonia, heart disease, restenosis, atherosclerosis, stoke, angina, and thrombosis. Furthermore, the protein may also be used to determine



biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:32 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2273 of SEQ ID NO:32, b is an integer of 15 to 2287, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:32, and where b is greater than or equal to a + 14.

### **FEATURES OF PROTEIN ENCODED BY GENE NO: 23**

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:  
 PGGGPCGNXWXPRGXREKKFVYSPNRLSLKVLALATAAASVTLTWIL  
 (SEQ ID NO: 145),  
 KEEQRRQAPGGQNGSWIVKKVWFACLA VMSFLGFILNLGARLIVQPQAALAS  
 RGLRGQGLPCETQVXKRTLPGAVGWL VHKGRRALSISRKSALVSLGVMYV  
 GPGKRPGVVRKHSLLVKMQAR (SEQ ID NO: 146),  
 KEEQRRQAPGGQNGSWIVKKVWFACLA VMSFLGFILNLGA (SEQ ID NO:  
 147), RLIVQPQAALASRGLRGQGLPCETQVXKRTLPGAVGWL V (SEQ ID  
 NO: 148), and/or  
 HKGRRALSISRKSALVSLGVMYVGPGKRPGVVRKHSLLVKMQAR (SEQ ID  
 NO: 149). Moreover, fragments and variants of these polypeptides (such as, for  
 example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%,



96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

When tested against U937 Myeloid cell lines, supernatants removed from cells containing this gene activated the GAS assay. Thus, it is likely that this gene activates myeloid cells, and to a lesser extent other cells, through the Jak-STAT signal transduction pathway. The gamma activating sequence (GAS) is a promoter element found upstream of many genes which are involved in the Jak-STAT pathway. The Jak-STAT pathway is a large, signal transduction pathway involved in the differentiation and proliferation of cells. Therefore, activation of the Jak-STAT pathway, reflected by the binding of the GAS element, can be used to indicate proteins involved in the proliferation and differentiation of cells.

It has been discovered that this gene is expressed highly and specifically in human adrenal gland tumor tissue, and to a significantly lesser extent in human thymus tissue.

Nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: cancer and other proliferative disorders of the adrenal gland. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the adrenal gland, expression of this gene at significantly higher or lower levels may be detected in certain tissues (e.g., adrenal gland, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 73 as residues:



Arg-25 to Gly-31, Ala-95 to Val-100. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution specifically in adrenal gland tumor tissue suggests that that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of cancer and other proliferative disorders of the adrenal gland, as well as cancers of other tissues where expression has been observed. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:33 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 674 of SEQ ID NO:33, b is an integer of 15 to 688, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:33, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 24**

The translation product of this gene shares sequence homology with mammalian Pig-L proteins and the yeast homolog, GPI12 or N-acetylglucosaminylphosphatidylinositol deacetylase, which are thought to be important in glycosylphosphatidylinositol biosynthesis (See, e.g., Genbank Accession Nos.: dbj|BAA74775.1|(AB017165) and gnl|PID|d1021712; all references available through these accessions are hereby incorporated in their entirety herein).

Glycosylphosphatidylinositol (GPI) is used as a membrane anchor by many eukaryotic cell-surface proteins. The second step of GPI biosynthesis is de-N-acetylation of N-acetylglucosaminylphosphatidylinositol (GlcNAc-PI). The activity of de-N-acetylases that act on the GlcNAc moiety, are enhanced by metal ions, in



particular  $Mn^{2+}$  and  $Ni^{2+}$ . On transfection into mammalian PIG-L-deficient cells, the yeast homolog, GPI12, restored the cell-surface expression of GPI-anchored proteins and GlcNAc-PI de-N-acetylase activity. The disruption of the gene caused lethality in *S. cerevisiae*, (see, e.g., Watanabe et al., J. Biochem.339:185-192 (1999), which is hereby incorporated in its entirety by reference herein). Based on the sequence similarity, the translation product of this clone is expected to share at least some biological activities with GlcNAc-PI de-N-acetylase proteins. Such activities are known in the art, some of which are described elsewhere herein. For example, one such assay is described in Kinoshita et al., J Biochem (Tokyo) 122:251-7 (1997), incorporated herein by reference.

The gene encoding the disclosed cDNA is thought to reside on chromosome 17. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 17.

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 137 - 153 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 154 - 192 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type Ia membrane proteins.

It has been discovered that this gene is expressed primarily in salivary gland and colon tissues, and to a lesser extent in microvascular endothelial cells, heart and placental tissues.

Nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: disorders caused by glycosylphosphatidylinositol deficiency. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the salivary gland or colon, expression of this gene at significantly higher or lower levels may be detected in certain tissues (e.g., salivary glands, gastrointestinal, cancerous and wounded tissues) or bodily fluids (e.g., saliva, lymph, serum, plasma, urine, synovial fluid or spinal fluid) taken



from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively  
 5 consist of, one or more immunogenic epitopes shown in SEQ ID NO: 74 as residues: Asp-20 to Gly-32. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution and homology to PIG-L and N-acetylglucosaminyl-phosphatidylinositol deacetylase suggests that polynucleotides and polypeptides  
 10 corresponding to this gene would be useful for diagnosis and treatment of disorders caused by glycosylphosphatidylinositol deficiency that interferes with the GPI-anchored surface protein functions. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to  
 15 its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are  
 20 related to SEQ ID NO:34 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the  
 25 general formula of a-b, where a is any integer between 1 to 981 of SEQ ID NO:34, b is an integer of 15 to 995, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:34, and where b is greater than or equal to a + 14.

30 **FEATURES OF PROTEIN ENCODED BY GENE NO: 25**



In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, the following amino acid sequence:

HIIFFRKWSTLAFIIPYSSVSGIISIASFMSVASEIASLVFLRKNTTFWSRNSSGRG  
VQS (SEQ ID NO: 150). Moreover, fragments and variants of these polypeptides

5 (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are  
10 also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

It has been discovered that this gene is expressed primarily in testes tissue.

Nucleic acids of the invention are useful as reagents for differential  
identification of the tissue(s) or cell type(s) present in a biological sample and for  
15 diagnosis of the following diseases and conditions: male infertility; testicular dysfunction. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the reproductive system, expression of this gene at significantly higher or lower levels  
20 may be detected in certain tissues (e.g., reproductive, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

25 The tissue distribution in testes tissue that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of male reproductive dysfunction. Enhanced expression of this gene product in testes suggests that it may play a key role in testicular function or in male fertility. Alternately, however, expression of many gene products is enhanced in testes nonspecifically,  
30 even if that gene product normally performs functions at alternate sites within the body. Thus, particularly since this represents a secreted protein product, it may play roles in many physiological processes. Furthermore, the tissue distribution that



polynucleotides and polypeptides corresponding to this gene are useful for the treatment and diagnosis of conditions concerning proper testicular function (e.g. endocrine function, sperm maturation), as well as cancer. Therefore, this gene product is useful in the treatment of male infertility and/or impotence. This gene product is also useful in assays designed to identify binding agents, as such agents (antagonists) are useful as male contraceptive agents. Similarly, the protein is believed to be useful in the treatment and/or diagnosis of testicular cancer. The testes are also a site of active gene expression of transcripts that may be expressed, particularly at low levels, in other tissues of the body. Therefore, this gene product may be expressed in other specific tissues or organs where it may play related functional roles in other processes, such as hematopoiesis, inflammation, bone formation, and kidney function, to name a few possible target indications. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:35 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 751 of SEQ ID NO:35, b is an integer of 15 to 765, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:35, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 26**

In specific embodiments, polypeptides of the invention comprise, or alternatively consists of, an amino acid sequence selected from the group:

VLCGPGAATRKGSQLNPAVASPAFHPGFFSLSNLGSSYSSSNTMYSCPSEPL  
HRLSPLPKETPLLSSPSPTXPSQPAELWFIFCIRVKGHLPCQSTPTLPLQSSEMSS



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It has been discovered that this gene is expressed primarily in neutrophils.

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inflammation, extravasation, tissue destruction, and recruitment of various cell types. Several of these potential roles would be undesirable, and therefore antagonists of this protein could prove beneficial in such instances. Alternately, expression of this gene product by neutrophils may be diagnostic of a more general function in

5 hematopoiesis, including effects on the proliferation, survival, differentiation, or activation of any or all hematopoietic cell lineages. Potential involvement of this protein in tissue extravasation may also have implications for the treatment of cancer and metastasis. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

10 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:36 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence

15 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 728 of SEQ ID NO:36, b is an integer of 15 to 742, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:36, and where b is greater than or equal to a

20 + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 27**

25 In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequences:  
 TSSPQRRLPAGPRPPTVEPPAEPPAEVPPSGTPPPPSTSEPLSRRRP (SEQ ID NO: 155). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the

30 polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by



the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise, or alternatively consist of, the following amino acid sequence:

TSSPQRRLPAGPRPPTVEPPAEPPAEVPPSGTPPPPSTSEPLSRRRPMWGFRLLR  
SPLLLLLLPQLGIGNASSCSQARTMNPGGSGGARCSLSAEVRRRQCLQLSTVP  
GAXPQRXNELLLLAAAGEGLERQDLPGDPAKEEPQPPPQHHVLYFPGDVQN  
YHEIMTRHPENYQWENWSLENVATILAHFRPNSYIWVIKCSRMHLHXFSCYD  
NFVKSNMFGAPEHNTDFGAFKHLVNAFNLSQNSLSKKSLNVWNKDSI  
ASNCRSSPSHTTNGCQGEKVRTCEKSDESAMSFYPPSLNDASFTLIGFSKGCV  
XLNQLLFELKEAKKDKNIDAFIKSIRTMYWLDGGHSGGSNTWVTYPEVLKEF  
AQTGIIVHHTVTPYQVRDPMRSWIGKEXKKFVQILGDLGMQVTSQIHFTKEA

P SIENHFRVHEVF (SEQ ID NO: 156). Moreover, fragments and variants of these polypeptides (such as, for example, fragments as described herein, polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to these polypeptides and polypeptides encoded by the polynucleotide which hybridizes, under stringent conditions, to the polynucleotide encoding these polypeptides, or the complement thereof are encompassed by the invention. Antibodies that bind polypeptides of the invention are also encompassed by the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The gene encoding the disclosed cDNA is thought to reside on chromosome 2. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 2.

It has been discovered that this gene is expressed primarily in B-cells, melanocytes, skeletal muscle, and a variety of other tissues and cell types.

Nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: melanoma, immunodeficiency, tissue necrosis, lymphomas, auto-immunities, cancer, metastasis, anemias (leukemia) and other hematopoietic disorders. Similarly, polypeptides and antibodies directed to



those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the skin and immune system, expression of this gene at significantly higher or lower levels may be detected in certain tissues (e.g., skin, immune, musculo-skeletal, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

Preferred polypeptides of the present invention comprise, or alternatively consist of, one or more immunogenic epitopes shown in SEQ ID NO: 77 as residues: Arg-31 to Gly-37, Val-49 to Cys-54. Polynucleotides encoding said polypeptides are also encompassed by the invention.

The tissue distribution in skin and immune tissues that polynucleotides and polypeptides corresponding to this gene would be useful for the diagnosis, detection, prevention and/or treatment of melanoma and other skin cancers, as well as immune disorders including leukemias, lymphomas, auto-immunities, immunodeficiencies (e.g., AIDS), immuno-suppressive conditions (e.g., transplantation) and hematopoietic disorders. In addition this gene product may be applicable in conditions of general microbial infection, inflammation and especially, cancer. Representative uses are described in the "Biological Activity", "Hyperproliferative Disorders", "Infectious Disease", and "Regeneration" sections below, in Example 11, 19, and 20, and elsewhere herein. Briefly, the protein is useful in detecting, treating, and/or preventing congenital disorders (i.e., nevi, moles, freckles, Mongolian spots, hemangiomas, port-wine syndrome), integumentary tumors (i.e., keratoses, Bowen's disease, basal cell carcinoma, squamous cell carcinoma, malignant melanoma, Paget's disease, mycosis fungoides, and Kaposi's sarcoma), injuries and inflammation of the skin (i.e., wounds, rashes, prickly heat disorder, psoriasis, dermatitis), atherosclerosis, urticaria, eczema, photosensitivity, autoimmune disorders (i.e., lupus erythematosus, vitiligo, dermatomyositis, morphea, scleroderma, pemphigoid, and pemphigus), keloids, striae, erythema, petechiae, purpura, and xanthelasma. In addition, such disorders may predispose increased susceptibility to viral and bacterial infections of the skin (i.e., cold sores, warts, chickenpox, molluscum contagiosum, herpes zoster,



(i.e., rheumatoid arthritis, lupus, scleroderma, dermatomyositis, etc.), dwarfism, spinal deformation, joint abnormalities, and chondrodysplasias (i.e., spondyloepiphyseal dysplasia congenita, familial osteoarthritis, Atelosteogenesis type II, metaphyseal chondrodysplasia type Schmid). Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and immunotherapy targets for the above listed tumors and tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:37 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2736 of SEQ ID NO:37, b is an integer of 15 to 2750, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:37, and where b is greater than or equal to a + 14.



**Table 1**

Gene No.	cDNA Clone ID	ATCC Deposit No.:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
1	HTDAA93	203858 03/18/99	pSport1	11	3191	1	3147	80	80	51	1	31	32	761
2	HWIAE49	203858 03/18/99	pCMVSPORT 3.0	12	1104	1	1104	106	106	52	1	23	24	305
3	HLWAX74	203858 03/18/99	pCMVSPORT 3.0	13	1927	8	1927	340	340	53	1	54	55	379
3	HLWAX74	203858 03/18/99	pCMVSPORT 3.0	38	1538	31	1538	375	375	78	1	19	20	292
3	HPJCX13	PTA-181 06/07/99	Uni-ZAP XR	39	5065	1643	5065	1830	1830	79	1	15	16	65
3	HNHCT15	203570 01/11/99	Uni-ZAP XR	40	4709	203	4709	1449	1449	80	1	30	31	1010
4	HE9RJ42	203858 03/18/99	Uni-ZAP XR	14	847	1	847	32	32	54	1	19	20	228
5	HDPAS92	203858 03/18/99	pCMVSPORT 3.0	15	2175	1	2175	130	130	55	1	31	32	552
6	HATDF29	203858 03/18/99	Uni-ZAP XR	16	1355	1	1355	143	143	56	1	30	31	385



Gene No.	cDNA Clone ID	ATCC Deposit No.:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
7	HWLHH15	203858 03/18/99	pSport1	17	2178	1	2178	77	77	57	1	19	20	190
8	HBXFL29	203858 03/18/99	ZAP Express	18	2229	376	2210	560	560	58	1	31	32	57
9	HKGBF67	203858 03/18/99	pSport1	19	1514	1	1514	101	101	59	1	23	24	443
9	HTEOF33	PTA-623 09/02/99	Uni-ZAP XR	41	2248	1	2248	314	314	81	1	23	24	120
10	HWHGP71	203858 03/18/99	pCMVSPORT 3.0	20	1021	1	1021	389	389	60	1	51	52	211
10	HWHGP71	203858 03/18/99	pCMVSPORT 3.0	42	1037	1	1037	394	394	82	1	18	19	77
11	HLWCU38	203858 03/18/99	pCMVSPORT 3.0	21	1859	337	1196	490	490	61	1	34	35	151
12	HMTAX46	203858 03/18/99	pCMVSPORT 3.0	22	1494	1	1494	26	26	62	1	20	21	118
13	HIBEU15	203858 03/18/99	Other	23	2105	64	2105	122	122	63	1	28	29	322
13	HIBEU15	203858 03/18/99	Other	43	2102	64	2102	84	84	83	1	40	41	256
14	HDPQV66	203858 03/18/99	pCMVSPORT 3.0	24	1290	1	1290	346	346	64	1	18	19	41



Gene No.	cDNA Clone ID	ATCC Deposit No.:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
15	HFXGW52	203858 03/18/99	Lambda ZAP II	25	1728	1	1728	165	165	65	1	42	43	152
16	HHEQR55	203858 03/18/99	pCMVSPORT 3.0	26	1569	1	1569	269	269	66	1	24	25	45
17	HNHNW84	203858 03/18/99	Uni-ZAP XR	27	1058	1	1058	35	35	67	1	34	35	72
18	HKAFH74	203858 03/18/99	pCMVSPORT 2.0	28	1353	1	1353	99	99	68	1	33	34	362
18	HKAFH74	203858 03/18/99	pCMVSPORT 2.0	44	1362	1	1362	104	104	84	1	33	34	61
19	HCUGE72	203858 03/18/99	ZAP Express	29	1078	1	1068	201	201	69	1	41	42	103
19	HCUFM70	209277 09/18/99	ZAP Express	45	390	1	390	197	197	85	1			23
20	HTEQI22	203858 03/18/99	Uni-ZAP XR	30	2412	873	2412	883	883	70	1	30	31	90
20	HTEQI22	203858 03/18/99	Uni-ZAP XR	46	1546	1	1536	19	19	86	1	20	21	90
20	HJBIC101	209745 04/07/98	pBluescript SK-	47	1643	1	1643	114	114	87	1	20	21	90
21	HDPYE41	203858 03/18/99	pCMVSPORT 3.0	31	1736	1	1736	256	256	71	1	19	20	43



Gene No.	cDNA Clone ID	ATCC Deposit No.:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
22	HDTII23	203858 03/18/99	pCMVSPORT 2.0	32	2287	109	2287	181	181	72	1	25	26	53
23	HATCM08	203858 03/18/99	Uni-ZAP XR	33	688	1	688	315	315	73	1	23	24	105
23	HATCM08	203858 03/18/99	Uni-ZAP XR	48	652	21	652	160	160	88	1	18	19	25
23	HTSFV18	203918 04/08/99	pBluescript	49	1093	1	1093	134	134	89	1	42	43	50
24	HAMFL84	203858 03/18/99	pCMVSPORT 3.0	34	995	1	995	9	9	74	1	18	19	192
25	HTELW37	203858 03/18/99	Uni-ZAP XR	35	765	1	765	243	243	75	1	20	21	56
26	HNGOU56	203858 03/18/99	Uni-ZAP XR	36	742	1	742	317	317	76	1	23	24	59
27	HOUHD63	203858 03/18/99	Uni-ZAP XR	37	2750	17	2750	144	144	77	1	22	23	385
27	HPJBF63	PTA-987 11/24/99	Uni-ZAP XR	50	2752	1	2752	151	151	90	1	22	23	385



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herein. Such methods include preparing probes or primers from the disclosed sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.

Also provided in the present invention are allelic variants, orthologs, and/or species homologs. Procedures known in the art can be used to obtain full-length genes, allelic variants, splice variants, full-length coding portions, orthologs, and/or species homologs of genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or a deposited clone, using information from the sequences disclosed herein or the clones deposited with the ATCC. For example, allelic variants and/or species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or the desired homologue.

Table 2 summarizes the expression profile of polynucleotides corresponding to the clones disclosed in Table 1. The first column provides a unique clone identifier, "Clone ID", for a cDNA clone related to each contig sequence disclosed in Table 1. Column 2, "Library Codes" shows the expression profile of tissue and/or cell line libraries which express the polynucleotides of the invention. Each Library Code in column 2 represents a tissue/cell source identifier code corresponding to the Library Code and Library description provided in Table 4. Expression of these polynucleotides was not observed in the other tissues and/or cell libraries tested. One of skill in the art could routinely use this information to identify tissues which show a predominant expression pattern of the corresponding polynucleotide of the invention or to identify polynucleotides which show predominant and/or specific tissue expression.

Table 3, column 1, provides a nucleotide sequence identifier, "SEQ ID NO:X," that matches a nucleotide SEQ ID NO:X disclosed in Table 1, column 5. Table 3, column 2, provides the chromosomal location, "Cytologic Band or Chromosome," of polynucleotides corresponding to SEQ ID NO:X. Chromosomal location was determined by finding exact matches to EST and cDNA sequences contained in the NCBI (National Center for Biotechnology Information) UniGene database. Given a presumptive chromosomal location, disease locus association was



determined by comparison with the Morbid Map, derived from Online Mendelian Inheritance in Man (Online Mendelian Inheritance in Man, OMIM™. McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD) 2000. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>). If the putative chromosomal location of the Query overlapped with the chromosomal location of a Morbid Map entry, the OMIM reference identification number of the morbid map entry is provided in Table 3, column 3, labelled "OMIM ID." A key to the OMIM reference identification numbers is provided in Table 5.

Table 4 provides a key to the Library Code disclosed in Table 2. Column 1 provides the Library Code disclosed in Table 2, column 2. Column 2 provides a description of the tissue or cell source from which the corresponding library was derived.

Table 5 provides a key to the OMIM reference identification numbers disclosed in Table 3, column 3. OMIM reference identification numbers (Column 1) were derived from Online Mendelian Inheritance in Man (Online Mendelian Inheritance in Man, OMIM. McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine, (Bethesda, MD) 2000. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>). Column 2 provides diseases associated with the cytologic band disclosed in Table 3, column 2, as determined using the Morbid Map database.



Table 2

Clone ID	Library Codes
HTDAA93	H0009 H0046 H0144 H0191 H0264 H0441 H0477 H0486 H0494 H0521 H0542 H0560 H0561 H0581 H0587 H0617 H0635 H0638 H0656 H0663 H0670 H0673 L0438 L0439 L0655 L0659 L0665 L0667 L0698 L0754 L0763 L0766 L0773 L0776 L0777 L0779 L0783 L0806 S0002 S0278 S0328 S0374 S0378 T0003
HWJAE49	H0014 H0494 H0587 H0602 H0661 H0670 H0690 L0376 L0657 L0794 L0806
HLWAX74	H0014 H0036 H0040 H0046 H0063 H0144 H0156 H0178 H0331 H0357 H0393 H0415 H0539 H0551 H0553 H0555 H0561 H0574 H0581 H0616 H0624 H0667 S0026 S0114 S0374 S0378
HE9RJ42	H0144 L0747
HDPAS92	H0041 H0083 H0135 H0255 H0267 H0295 H0341 H0413 H0422 H0423 H0497 H0519 H0521 H0529 H0545 H0547 H0551 H0580 H0585 H0617 H0618 H0633 H0665 L0372 L0439 L0640 L0659 L0663 L0666 L0744 L0757 L0789 L0800 L0803 S0038 S0250 S0420
HATDF29	H0038 H0144 H0156 H0179 H0253 H0271 H0393 H0402 H0485 H0486 H0494 H0521 H0555 H0574 H0575 H0581 H0618 H0659 H0661 L0363 L0438 L0518 L0589 L0596 L0599 L0665 L0731 L0745 L0748 L0751 L0754 L0756 L0757 L0758 L0759 L0763 L0766 L0771 L0775 L0776 L0777 L0779 L0805 S0002 S0132 S0136 S0142 S0278 S0344 S0360 S0374 S0376 S0398 S0412 S0432 T0004 T0023 T0041 T0110
HWLHH15	S0354
HBXFL29	H0014 H0032 H0038 H0050 H0052 H0131 H0370 H0375 H0412 H0413 H0416 H0435 H0438 H0521 H0547 H0551 H0574 H0581 H0594 H0596 H0632 H0663 H0674 L0438 L0439 L0455 L0471 L0592 L0608 L0638 L0662 L0731 L0754 L0755 L0758 L0766 L0769 L0771 L0774 L0775 L0777 L0803 S0010 S0182 S0194 S0222 S0242 S0346 S0360 S0380 S6026 T0110
HKGBF67	H0036 H0038 H0393 H0412 H0428 H0494 H0520 H0538 H0545 H0555 H0599 H0612 H0616 H0674 L0021 L0471 L0637 L0665 L0731 L0740 L0749 L0752 L0759 L0774 L0776 L0789 L0803 L0804 L0805 L0806 S0010 S0358 S0360 T0041
HWHGP71	H0457 H0521 H0586 H0634 L0745 L0746
HLWCU38	H0009 H0014 H0023 H0038 H0040 H0046 H0050 H0052 H0059 H0063 H0083 H0090 H0123 H0144 H0251 H0265 H0266 H0271 H0284 H0341 H0411 H0413 H0421 H0435 H0445 H0457 H0484 H0494 H0497 H0509 H0519 H0521 H0538 H0539 H0544 H0549 H0551 H0553 H0574 H0581 H0583 H0624 H0627 H0641 H0648 H0657 H0659 H0660 H0674 L0438 L0439 L0480 L0518 L0527 L0589 L0595 L0602 L0608 L0631 L0641 L0648 L0651 L0659 L0662 L0663 L0664 L0665 L0666 L0731 L0738 L0740 L0744 L0747 L0748 L0749 L0750 L0751 L0753 L0754 L0757 L0758 L0759 L0761 L0763 L0764 L0766 L0767 L0768 L0769 L0770 L0771 L0773 L0774 L0775 L0776 L0777 L0779 L0780 L0789 L0794 L0800 L0803 L0804 L0805 L0809 S0002 S0003 S0026 S0036 S0040 S0052 S0126 S0142 S0152 S0212 S0278 S0328 S0330 S0344 S0354 S0358 S0374 S0376 S0420 S0426 S0434 S3014 S6028
HMTAX46	H0040 H0069 H0085 H0179 H0264 H0271 H0284 H0309 H0412 H0436 H0457 H0518 H0522 H0543 H0555 H0556 H0575 H0581 H0587 H0595 H0688 L0021 L0439 L0662 L0663 L0664 L0666 L0667 L0731 L0740 L0754 L0766 L0777 L0779 L0783 L0794 S0114 S0150 S0356 S0358



	S0420 S6016
HIBEU15	H0052 H0264 H0441 H0455 H0566 H0572 L0439 L0747 L0770 T0010
HDPQV66	H0031 H0050 H0263 H0294 H0428 H0445 H0486 H0521 H0522 H0598 H0617 H0661 H0664 H0667 H0688 L0439 L0471 L0483 L0518 L0602 L0608 L0642 L0659 L0664 L0665 L0731 L0742 L0747 L0752 L0754 L0756 L0758 L0761 L0764 L0766 L0770 L0771 L0776 L0779 L0800 L0805 L0809 S0003 S0010 S0049 S0134 S0330 S0356
HFXGW52	S0001
HHEQR55	H0543
HNHNW84	S0216
HKAFH74	H0083 H0255 H0427 H0494 H0521 H0539 H0545 H0561 H0587 H0593 H0617 H0622 H0638 L0097 L0382 L0439 L0646 L0648 L0655 L0657 L0659 L0665 L0666 L0747 L0751 L0754 L0759 L0763 L0764 L0766 L0769 L0770 L0772 L0777 L0780 L0789 L0794 L0800 L0803 L0804 L0809 S0328 S0354 S0358 S0376 S0418 S0448
HCUGE72	H0052 H0087 H0181 H0188 H0266 H0352 H0402 H0441 H0546 H0555 H0595 H0606 H0616 H0617 H0674 H0684 L0438 L0527 L0565 L0601 L0657 L0659 L0665 L0731 L0744 L0747 L0748 L0751 L0753 L0754 L0756 L0758 L0769 L0770 L0774 L0776 L0777 L0779 L0783 L0786 S0031 S0050 S0051 S0053 S0360 S0468 T0023
HTEQI22	H0013 H0031 H0038 H0046 H0052 H0144 H0252 H0261 H0265 H0369 H0374 H0428 H0494 H0519 H0521 H0560 H0615 H0616 H0624 H0628 H0634 H0650 H0662 H0667 H0674 H0690 L0363 L0438 L0439 L0536 L0591 L0593 L0596 L0608 L0646 L0653 L0659 L0662 L0731 L0740 L0747 L0748 L0749 L0750 L0758 L0761 L0764 L0766 L0768 L0769 L0773 L0776 L0777 L0789 L0791 L0793 L0803 L0806 S0006 S0010 S0152 S0278 S0358 S0418 S0458 S6026 T0002 T0041 T0042 T0067
HDPYE41	H0521 S0344
HDTII23	H0486 H0547 H0575 L0592 L0599 L0607 L0759 L0761 S0422
HATCM08	H0087 H0156 L0517
HAMFL84	H0266 H0478 H0560 L0438 L0731 L0747 L0749 L0752 L0755 L0759 L0764 L0771 L0779 L0805 S0278
HTELW37	H0616
HNGOU56	S0428
HOUHD63	H0266 H0351 H0381 H0435 H0486 H0509 H0542 H0543 H0591 H0593 H0596 H0622 H0672 H0673 H0676 H0687 L0194 L0372 L0439 L0485 L0637 L0664 L0731 L0740 L0745 L0752 L0756 L0757 L0766 L0776 L0777 L0779 L0790 L0803 L0804 L0806 S0007 S0152 S0192 S0276 S0294 S0330 S0342 S0344 S6024 T0042



### Table 3

SEQ ID	Cytologic Band or	OMIM ID
NO: X	Chromosome:	
12	19q13	109560 205900 600652 600757



### Table 4

Library Code	Library Description
H0009	Human Fetal Brain
H0013	Human 8 Week Whole Embryo
H0014	Human Gall Bladder
H0023	Human Fetal Lung
H0031	Human Placenta
H0032	Human Prostate
H0036	Human Adult Small Intestine
H0038	Human Testes
H0040	Human Testes Tumor
H0041	Human Fetal Bone
H0046	Human Endometrial Tumor
H0050	Human Fetal Heart
H0052	Human Cerebellum
H0059	Human Uterine Cancer
H0063	Human Thymus
H0069	Human Activated T-Cells
H0083	HUMAN JURKAT MEMBRANE BOUND POLYSOMES
H0085	Human Colon
H0087	Human Thymus
H0090	Human T-Cell Lymphoma
H0123	Human Fetal Dura Mater
H0131	LNCAP + 0.3nM R1881
H0135	Human Synovial Sarcoma
H0144	Nine Week Old Early Stage Human
H0156	Human Adrenal Gland Tumor
H0178	Human Fetal Brain
H0179	Human Neutrophil
H0181	Human Primary Breast Cancer
H0188	Human Normal Breast
H0191	Human Activated Macrophage (LPS), thiour
H0251	Human Chondrosarcoma
H0252	Human Osteosarcoma
H0253	Human adult testis, large inserts
H0255	breast lymph node CDNA library
H0261	H. cerebellum, Enzyme subtracted
H0263	human colon cancer
H0264	human tonsils
H0265	Activated T-Cell (12hs)/Thiouridine labelledEco
H0266	Human Microvascular Endothelial Cells, fract. A
H0267	Human Microvascular Endothelial Cells, fract. B
H0271	Human Neutrophil, Activated
H0284	Human OB MG63 control fraction I
H0294	Amniotic Cells - TNF induced
H0295	Amniotic Cells - Primary Culture
H0309	Human Chronic Synovitis
H0331	Hepatocellular Tumor
H0341	Bone Marrow Cell Line (RS4,11)
H0351	Glioblastoma



H0352	wilm's tumor
H0357	H. Normalized Fetal Liver, II
H0369	H. Atrophic Endometrium
H0370	H. Lymph node breast Cancer
H0374	Human Brain
H0375	Human Lung
H0381	Bone Cancer
H0393	Fetal Liver, subtraction II
H0402	CD34 depleted Buffy Coat (Cord Blood), re-excision
H0411	H Female Bladder, Adult
H0412	Human umbilical vein endothelial cells, IL-4 induced
H0413	Human Umbilical Vein Endothelial Cells, uninduced
H0415	H. Ovarian Tumor, II, OV5232
H0416	Human Neutrophils, Activated, re-excision
H0421	Human Bone Marrow, re-excision
H0422	T-Cell PHA 16 hrs
H0423	T-Cell PHA 24 hrs
H0427	Human Adipose
H0428	Human Ovary
H0435	Ovarian Tumor 10-3-95
H0436	Resting T-Cell Library,II
H0438	H. Whole Brain #2, re-excision
H0441	H. Kidney Cortex, subtracted
H0445	Spleen, Chronic lymphocytic leukemia
H0455	H. Striatum Depression, subt
H0457	Human Eosinophils
H0477	Human Tonsil, Lib 3
H0478	Salivary Gland, Lib 2
H0484	Breast Cancer Cell line, angiogenic
H0485	Hodgkin's Lymphoma I
H0486	Hodgkin's Lymphoma II
H0494	Keratinocyte
H0497	HEL cell line
H0509	Liver, Hepatoma
H0518	pBMC stimulated w/ poly I/C
H0519	NTERA2, control
H0520	NTERA2 + retinoic acid, 14 days
H0521	Primary Dendritic Cells, lib 1
H0522	Primary Dendritic cells,frac 2
H0529	Myeloid Progenitor Cell Line
H0538	Merkel Cells
H0539	Pancreas Islet Cell Tumor
H0542	T Cell helper I
H0543	T cell helper II
H0544	Human endometrial stromal cells
H0545	Human endometrial stromal cells-treated with progesterone
H0546	Human endometrial stromal cells-treated with estradiol
H0547	NTERA2 teratocarcinoma cell line+retinoic acid (14 days)
H0549	H. Epididymus, caput & corpus
H0551	Human Thymus Stromal Cells
H0553	Human Placenta
H0555	Rejected Kidney, lib 4
H0556	Activated T-cell(12h)/Thiouridine-re-excision



H0560	KMH2
H0561	L428
H0566	Human Fetal Brain,normalized c50F
H0572	Human Fetal Brain, normalized AC5002
H0574	Hepatocellular Tumor, re-excision
H0575	Human Adult Pulmonary,re-excision
H0580	Dendritic cells, pooled
H0581	Human Bone Marrow, treated
H0583	B Cell lymphoma
H0585	Activated T-Cells,12 hrs,re-excision
H0586	Healing groin wound, 6.5 hours post incision
H0587	Healing groin wound, 7.5 hours post incision
H0591	Human T-cell lymphoma,re-excision
H0593	Olfactory epithelium,nasalcavity
H0594	Human Lung Cancer,re-excision
H0595	Stomach cancer (human),re-excision
H0596	Human Colon Cancer,re-excision
H0598	Human Stomach,re-excision
H0599	Human Adult Heart,re-excision
H0602	Healing Abdomen Wound,21&29 days post incision
H0606	Human Primary Breast Cancer,re-excision
H0612	H.Leukocytes, normalized cot 50 B
H0615	Human Ovarian Cancer Reexcision
H0616	Human Testes, Reexcision
H0617	Human Primary Breast Cancer Reexcision
H0618	Human Adult Testes, Large Inserts, Reexcision
H0622	Human Pancreas Tumor, Reexcision
H0624	12 Week Early Stage Human II, Reexcision
H0627	Saos2 Cells, Vitamin D3 Treated
H0628	Human Pre-Differentiated Adipocytes
H0632	Hepatocellular Tumor,re-excision
H0633	Lung Carcinoma A549 TNFalpha activated
H0634	Human Testes Tumor, re-excision
H0635	Human Activated T-Cells, re-excision
H0638	CD40 activated monocyte dendridic cells
H0641	LPS activated derived dendritic cells
H0648	Ovary, Cancer: (4004562 B6) Papillary Serous Cystic Neoplasm, Low Malignant Pot
H0650	B-Cells
H0656	B-cells (unstimulated)
H0657	B-cells (stimulated)
H0659	Ovary, Cancer (15395A1F): Grade II Papillary Carcinoma
H0660	Ovary, Cancer: (15799A1F) Poorly differentiated carcinoma
H0661	Breast, Cancer: (4004943 A5)
H0662	Breast, Normal: (4005522B2)
H0663	Breast, Cancer: (4005522 A2)
H0664	Breast, Cancer: (9806C012R)
H0665	Stromal cells 3.88
H0667	Stromal cells(HBM3.18)
H0670	Ovary, Cancer(4004650 A3): Well-Differentiated Micropapillary Serous Carcinoma
H0672	Ovary, Cancer: (4004576 A8)
H0673	Human Prostate Cancer, Stage B2, re-excision
H0674	Human Prostate Cancer, Stage C, re-excision



H0676	Colon, Cancer: (9808C064R)-total RNA
H0684	Ovarian cancer, Serous Papillary Adenocarcinoma
H0687	Human normal ovary(#9610G215)
H0688	Human Ovarian Cancer(#9807G017)
H0690	Ovarian Cancer, # 9702G001
L0021	Human adult (K.Okubo)
L0097	Subtracted human retinal pigment epithelium (RPE)
L0194	Human pancreatic cancer cell line Patu 8988t
L0363	NCI CGAP GC2
L0372	NCI CGAP Co12
L0376	NCI CGAP Lar1
L0382	NCI CGAP Pr25
L0438	normalized infant brain cDNA
L0439	Soares infant brain 1NIB
L0455	Human retina cDNA randomly primed sublibrary
L0471	Human fetal heart, Lambda ZAP Express
L0480	Stratagene cat#937212 (1992)
L0483	Human pancreatic islet
L0485	STRATAGENE Human skeletal muscle cDNA library, cat. #936215.
L0517	NCI CGAP Pr1
L0518	NCI CGAP Pr2
L0527	NCI CGAP Ov2
L0536	NCI CGAP Br4
L0565	Normal Human Trabecular Bone Cells
L0589	Stratagene fetal retina 937202
L0591	Stratagene HeLa cell s3 937216
L0592	Stratagene hNT neuron (#937233)
L0593	Stratagene neuroepithelium (#937231)
L0595	Stratagene NT2 neuronal precursor 937230
L0596	Stratagene colon (#937204)
L0599	Stratagene lung (#937210)
L0601	Stratagene pancreas (#937208)
L0602	Pancreatic Islet
L0607	NCI CGAP Lym6
L0608	Stratagene lung carcinoma 937218
L0631	NCI CGAP Br7
L0637	NCI CGAP Brn53
L0638	NCI CGAP Brn35
L0640	NCI CGAP Br18
L0641	NCI CGAP Co17
L0642	NCI CGAP Co18
L0646	NCI CGAP Co14
L0648	NCI CGAP Eso2
L0651	NCI CGAP Kid8
L0653	NCI CGAP Lu28
L0655	NCI CGAP Lym12
L0657	NCI CGAP Ov23
L0659	NCI CGAP Pan1
L0662	NCI CGAP Gas4
L0663	NCI CGAP Ut2
L0664	NCI CGAP Ut3
L0665	NCI CGAP Ut4
L0666	NCI CGAP Ut1



L0667	NCI CGAP CML1
L0698	Testis 2
L0731	Soares pregnant uterus NbHPU
L0738	Human colorectal cancer
L0740	Soares melanocyte 2NbHM
L0742	Soares adult brain N2b5HB55Y
L0744	Soares breast 3NbHBst
L0745	Soares retina N2b4HR
L0746	Soares retina N2b5HR
L0747	Soares fetal heart NbHH19W
L0748	Soares fetal liver spleen 1NFLS
L0749	Soares fetal liver spleen 1NFLS S1
L0750	Soares fetal lung NbHL19W
L0751	Soares ovary tumor NbHOT
L0752	Soares parathyroid tumor NbHPA
L0753	Soares pineal gland N3HPG
L0754	Soares placenta Nb2HP
L0755	Soares placenta 8to9weeks 2NbHP8to9W
L0756	Soares multiple sclerosis 2NbHMSP
L0757	Soares senescent fibroblasts NbHSF
L0758	Soares testis NHT
L0759	Soares total fetus Nb2HF8 9w
L0761	NCI CGAP CLL1
L0763	NCI CGAP Br2
L0764	NCI CGAP Co3
L0766	NCI CGAP GCB1
L0767	NCI CGAP GC3
L0768	NCI CGAP GC4
L0769	NCI CGAP Brn25
L0770	NCI CGAP Brn23
L0771	NCI CGAP Co8
L0772	NCI CGAP Co10
L0773	NCI CGAP Co9
L0774	NCI CGAP Kid3
L0775	NCI CGAP Kid5
L0776	NCI CGAP Lu5
L0777	Soares NhHMPu S1
L0779	Soares NFL T GBC S1
L0780	Soares NSF F8 9W OT PA P S1
L0783	NCI CGAP Pr22
L0786	Soares NbHFB
L0789	NCI CGAP Sub3
L0790	NCI CGAP Sub4
L0791	NCI CGAP Sub5
L0793	NCI CGAP Sub7
L0794	NCI CGAP GC6
L0800	NCI CGAP Co16
L0803	NCI CGAP Kid11
L0804	NCI CGAP Kid12
L0805	NCI CGAP Lu24
L0806	NCI CGAP Lu19
L0809	NCI CGAP Pr28
S0001	Brain frontal cortex



S0002	Monocyte activated
S0003	Human Osteoclastoma
S0006	Neuroblastoma
S0007	Early Stage Human Brain
S0010	Human Amygdala
S0026	Stromal cell TF274
S0031	Spinal cord
S0036	Human Substantia Nigra
S0038	Human Whole Brain #2 - Oligo dT > 1.5Kb
S0040	Adipocytes
S0049	Human Brain, Striatum
S0050	Human Frontal Cortex, Schizophrenia
S0051	Human Hypothalamus,Schizophrenia
S0052	neutrophils control
S0053	Neutrophils IL-1 and LPS induced
S0114	Anergic T-cell
S0126	Osteoblasts
S0132	Epithelial-TNF $\alpha$ and INF induced
S0134	Apoptotic T-cell
S0136	PERM TF274
S0142	Macrophage-oxLDL
S0150	LNCAP prostate cell line
S0152	PC3 Prostate cell line
S0182	Human B Cell 8866
S0192	Synovial Fibroblasts (control)
S0194	Synovial hypoxia
S0212	Bone Marrow Stromal Cell, untreated
S0216	Neutrophils IL-1 and LPS induced
S0222	H. Frontal cortex,epileptic,re-excision
S0242	Synovial Fibroblasts (IL1/TNF), subt
S0250	Human Osteoblasts II
S0276	Synovial hypoxia-RSF subtracted
S0278	H Macrophage (GM-CSF treated), re-excision
S0294	Larynx tumor
S0328	Palate carcinoma
S0330	Palate normal
S0342	Adipocytes,re-excision
S0344	Macrophage-oxLDL, re-excision
S0346	Human Amygdala,re-excision
S0354	Colon Normal II
S0356	Colon Carcinoma
S0358	Colon Normal III
S0360	Colon Tumor II
S0374	Normal colon
S0376	Colon Tumor
S0378	Pancreas normal PCA4 No
S0380	Pancreas Tumor PCA4 Tu
S0398	Testis, normal
S0412	Temporal cortex-Alzheimer, subtracted
S0418	CHME Cell Line,treated 5 hrs
S0420	CHME Cell Line,untreated
S0422	Mo7e Cell Line GM-CSF treated (1ng/ml)
S0426	Monocyte activated, re-excision



S0428	Neutrophils control, re-excision
S0432	Sinus piniformis Tumour
S0434	Stomach Normal
S0448	Larynx Normal
S0458	Thyroid Normal (SDCA2 No)
S0468	Ea.hy.926 cell line
S3014	Smooth muscle, serum induced,re-exc
S6016	H. Frontal Cortex, Epileptic
S6024	Alzheimers, spongy change
S6026	Frontal Lobe, Dementia
S6028	Human Manic Depression Tissue
T0002	Activated T-cells
T0003	Human Fetal Lung
T0004	Human White Fat
T0010	Human Infant Brain
T0023	Human Pancreatic Carcinoma
T0041	Jurkat T-cell G1 phase
T0042	Jurkat T-Cell, S phase
T0067	Human Thyroid
T0110	Human colon carcinoma (HCC) cell line, remake



**Table 5**

OMIM ID	OMIM Description
109560	Leukemia/lymphoma, B-cell, 3 (2)
205900	Anemia, Diamond-Blackfan (2)
600652	Deafness, autosomal dominant 4 (2)
600757	Orofacial cleft-3 (2)



The polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially purified using techniques described herein or otherwise known in the art, such as, for example, by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural, synthetic or recombinant sources using techniques described herein or otherwise known in the art, such as, for example, antibodies of the invention raised against the secreted protein.

The present invention provides a polynucleotide comprising, or alternatively consisting of, the nucleic acid sequence of SEQ ID NO:X, and/or a cDNA contained in ATCC deposit Z. The present invention also provides a polypeptide comprising, or alternatively, consisting of, the polypeptide sequence of SEQ ID NO:Y and/or a polypeptide encoded by the cDNA contained in ATCC deposit Z. Polynucleotides encoding a polypeptide comprising, or alternatively consisting of the polypeptide sequence of SEQ ID NO:Y and/or a polypeptide sequence encoded by the cDNA contained in ATCC deposit Z are also encompassed by the invention.

#### **Signal Sequences**

The present invention also encompasses mature forms of the polypeptide having the polypeptide sequence of SEQ ID NO:Y and/or the polypeptide sequence encoded by the cDNA in a deposited clone. Polynucleotides encoding the mature forms (such as, for example, the polynucleotide sequence in SEQ ID NO:X and/or the



polynucleotide sequence contained in the cDNA of a deposited clone) are also encompassed by the invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretary leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough  
 5 endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species of the protein. Further, it has long been known that cleavage specificity of a secreted protein is ultimately determined by the primary structure of  
 10 the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide.

Methods for predicting whether a protein has a signal sequence, as well as the cleavage point for that sequence, are available. For instance, the method of McGeoch, *Virus Res.* 3:271-286 (1985), uses the information from a short N-terminal  
 15 charged region and a subsequent uncharged region of the complete (uncleaved) protein. The method of von Heinje, *Nucleic Acids Res.* 14:4683-4690 (1986) uses the information from the residues surrounding the cleavage site, typically residues -13 to +2, where +1 indicates the amino terminus of the secreted protein. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of  
 20 these methods is in the range of 75-80%. (von Heinje, *supra.*) However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

In the present case, the deduced amino acid sequence of the secreted polypeptide was analyzed by a computer program called SignalP (Henrik Nielsen et  
 25 al., *Protein Engineering* 10:1-6 (1997)), which predicts the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis of the amino acid sequences of the secreted proteins described herein by this program provided the results shown in Table 1.

30 As one of ordinary skill would appreciate, however, cleavage sites sometimes vary from organism to organism and cannot be predicted with absolute certainty. Accordingly, the present invention provides secreted polypeptides having a sequence



shown in SEQ ID NO:Y which have an N-terminus beginning within 5 residues (i.e., + or - 5 residues) of the predicted cleavage point. Similarly, it is also recognized that in some cases, cleavage of the signal sequence from a secreted protein is not entirely uniform, resulting in more than one secreted species. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Moreover, the signal sequence identified by the above analysis may not necessarily predict the naturally occurring signal sequence. For example, the naturally occurring signal sequence may be further upstream from the predicted signal sequence. However, it is likely that the predicted signal sequence will be capable of directing the secreted protein to the ER. Nonetheless, the present invention provides the mature protein produced by expression of the polynucleotide sequence of SEQ ID NO:X and/or the polynucleotide sequence contained in the cDNA of a deposited clone, in a mammalian cell (e.g., COS cells, as described below). These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

### **Polynucleotide and Polypeptide Variants**

The present invention is directed to variants of the polynucleotide sequence disclosed in SEQ ID NO:X, the complementary strand thereto, and/or the cDNA sequence contained in a deposited clone.

The present invention also encompasses variants of the polypeptide sequence disclosed in SEQ ID NO:Y and/or encoded by a deposited clone.

"Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

The present invention is also directed to nucleic acid molecules which comprise, or alternatively consist of, a nucleotide sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for example, the nucleotide coding sequence in SEQ ID NO:X or the complementary strand thereto, the nucleotide coding sequence contained in a deposited cDNA clone or the



complementary strand thereto, a nucleotide sequence encoding the polypeptide of SEQ ID NO:Y, a nucleotide sequence encoding the polypeptide encoded by the cDNA contained in a deposited clone, and/or polynucleotide fragments of any of these nucleic acid molecules (e.g., those fragments described herein).

- 5 Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

The present invention is also directed to polypeptides which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 85%, 90%,  
 10 95%, 96%, 97%, 98%, 99% identical to, for example, the polypeptide sequence shown in SEQ ID NO:Y, the polypeptide sequence encoded by the cDNA contained in a deposited clone, and/or polypeptide fragments of any of these polypeptides (e.g., those fragments described herein).

By a nucleic acid having a nucleotide sequence at least, for example, 95%  
 15 "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the nucleic acid is identical to the reference sequence except that the nucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the polypeptide. In other words, to obtain a nucleic acid having a nucleotide sequence at least 95%  
 20 identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be an entire sequence shown in Table 1, the ORF (open reading frame), or any fragment specified  
 25 as described herein.

As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best  
 30 overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp.



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deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, an amino acid sequences shown in Table 1 (SEQ ID NO:Y) or to the amino acid sequence encoded by cDNA contained in a deposited clone can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245(1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window



If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not



matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

The variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. The authors of Ron et al., *J. Biol. Chem.* 268: 2984-2988 (1993), reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., *J. Biotechnology* 7:199-216 (1988).)

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (*J. Biol. Chem.* 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over



3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See, Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Thus, the invention further includes polypeptide variants which show substantial biological activity. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.



The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used.

- 5 (Cunningham and Wells, Science 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

- As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

- Besides conservative amino acid substitution, variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as, for example, an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification or (v) fusion of the polypeptide with another compound, such as albumin (including, but not limited to, recombinant albumin (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)). Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.



For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of the present invention having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a peptide or polypeptide to have an amino acid sequence which comprises the amino acid sequence of the present invention, which contains at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid substitutions. In specific embodiments, the number of additions, substitutions, and/or deletions in the amino acid sequence of the present invention or fragments thereof (e.g., the mature form and/or other fragments described herein), is 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, conservative amino acid substitutions are preferable.

### **Polynucleotide and Polypeptide Fragments**

The present invention is also directed to polynucleotide fragments of the polynucleotides of the invention.

In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence which: is a portion of that contained in a deposited clone, or encoding the polypeptide encoded by the cDNA in a deposited clone; is a portion of that shown in SEQ ID NO:X or the complementary strand thereto, or is a portion of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:Y. The nucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt,



and even more preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, or at least about 150 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in a deposited clone or the nucleotide sequence shown in SEQ ID NO:X. In this context "about" includes the particularly recited value, a value larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. These nucleotide fragments have uses that include, but are not limited to, as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, 500, 600, 2000 nucleotides) are preferred.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, 701-750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, or 2001 to the end of SEQ ID NO:X, or the complementary strand thereto, or the cDNA contained in a deposited clone. In this context "about" includes the particularly recited ranges, and ranges larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has biological activity. More preferably, these polynucleotides can be used as probes or primers as discussed herein. Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

In the present invention, a "polypeptide fragment" refers to an amino acid sequence which is a portion of that contained in SEQ ID NO:Y or encoded by the cDNA contained in a deposited clone. Protein (polypeptide) fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, from about amino acid number 1-20, 21-40,



41-60, 61-80, 81-100, 102-120, 121-140, 141-160, or 161 to the end of the coding region. Moreover, polypeptide fragments can be about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or values, and ranges or values larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Preferred polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also preferred.

Also preferred are polypeptide and polynucleotide fragments characterized by structural or functional domains, such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Polypeptide fragments of SEQ ID NO:Y falling within conserved domains are specifically contemplated by the present invention. Moreover, polynucleotides encoding these domains are also contemplated.

Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.



Preferably, the polynucleotide fragments of the invention encode a polypeptide which demonstrates a functional activity. By a polypeptide demonstrating a "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) polypeptide of invention protein. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a polypeptide of the invention for binding) to an antibody to the polypeptide of the invention], immunogenicity (ability to generate antibody which binds to a polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide of the invention.

The functional activity of polypeptides of the invention, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods.

For example, in one embodiment where one is assaying for the ability to bind or compete with full-length polypeptide of the invention for binding to an antibody of the polypeptide of the invention, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In another embodiment, where a ligand for a polypeptide of the invention identified, or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel



5 In addition, assays described herein (see Examples) and otherwise known in the art may routinely be applied to measure the ability of polypeptides of the invention and fragments, variants derivatives and analogs thereof to elicit related biological activity related to that of the polypeptide of the invention (either in vitro or in vivo). Other methods will be known to the skilled artisan and are within the scope of the invention.

The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide having an amino acid sequence of SEQ ID NO:Y, or an epitope of the polypeptide sequence encoded by a polynucleotide sequence contained in ATCC deposit No. Z or encoded by a polynucleotide that hybridizes to the complement of the sequence of SEQ ID NO:X or contained in ATCC deposit No. Z under stringent hybridization conditions or lower stringency hybridization conditions as defined supra. The present invention further encompasses polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:X), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to the complementary strand under stringent hybridization conditions or lower stringency hybridization conditions defined supra.

The term “epitopes,” as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An “immunogenic epitope,” as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies



described *infra*. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998- 4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross- reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985), further described in U.S. Patent No. 4,631,211).

In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe et al., Science 219:660-666 (1983)).

Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., *supra*; Wilson et al., *supra*; Chow et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle et al., J. Gen. Virol. 66:2347-2354 (1985). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to



an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., supra; Wilson et al., supra, and Bittle et al., *J. Gen. Virol.*, 66:2347-2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl- N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier- coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µg of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention comprising an immunogenic or antigenic epitope can be fused to other polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof), or albumin (including but not limited to recombinant



albumin (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)), resulting in chimeric polypeptides. Such fusion proteins may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., *Nature*, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., *J. Biochem.*, 270:3958-3964 (1995). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:8972- 897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni<sup>2+</sup> nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721;



5,834,252; and 5,837,458, and Patten et al., Curr. Opinion Biotechnol. 8:724-33 (1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308- 13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:X and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

### **Antibodies**

Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of SEQ ID NO:Y, and/or an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. In preferred embodiments, the immunoglobulin



molecules of the invention are IgG1. In other preferred embodiments, the immunoglobulin molecules of the invention are IgG4.

Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')<sub>2</sub>, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, sheep rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be



excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog,

5 ortholog, or homolog of a polypeptide of the present invention are included.

Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present

10 invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using

15 methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent

20 hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M,  $5 \times 10^{-5}$  M,  $10^{-5}$  M,  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M,  $10^{-8}$  M,  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, or  $10^{-15}$  M.

25 The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the

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epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes  
5 antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferably, antibodies of the present invention bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand  
10 binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described  
15 supra). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

The invention also features receptor-specific antibodies which both prevent  
20 ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but  
25 do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists  
30 for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No.



5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of



numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

5           The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of- interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal  
10       antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and  
15       potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term “monoclonal antibody” as used herein is not limited to antibodies produced through hybridoma technology. The term “monoclonal antibody” refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma  
30 technology are routine and well known in the art and are discussed in detail in the  
Examples (e.g., Example 16). In a non-limiting example, mice can be immunized  
with a polypeptide of the invention or a cell expressing such peptide. Once an



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gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic  
5 et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225;  
10 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and  
15 expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')<sub>2</sub> fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., BioTechniques 12(6):864-869 (1992); and Sawai et al., AJRI 34:26-  
20 34 (1995); and Better et al., Science 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., Methods in Enzymology 203:46-88 (1991); Shu et al., PNAS 90:7995-7999  
25 (1993); and Skerra et al., Science 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal  
30 antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. Immunol.



Methods 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entirety.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into



mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention.

Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, *Int. Rev. Immunol.* 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., *Bio/technology* 12:899-903 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using







generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well known in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino



acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423-42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may also be used (Skerra et al., Science 242:1038-1041 (1988)).

## 25 *Methods of Producing Antibodies*

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a



polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as



bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems  
 5 infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO,  
 10 BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole  
 15 recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., *Gene* 45:101 (1986); Cockett et al., *Bio/Technology* 8:2  
 20 (1990)).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which  
 25 direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., *EMBO J.* 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, *Nucleic  
 30 Acids Res.* 13:3101-3109 (1985); Van Heeke & Schuster, *J. Biol. Chem.* 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such



fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein.



Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt,



which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 5 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, 1993, TIB TECH 11(5):155-215; and hygro, which confers resistance to hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. 10 (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

15 The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in 20 culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second 25 vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic 30 free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.



Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., *supra*, and PCT publication WO 93/21232; EP 439,095; Naramura et al., *Immunol. Lett.* 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., *PNAS* 89:1428-1432 (1992); Fell et al., *J. Immunol.* 146:2446-2452(1991), which are incorporated by reference in their entireties.

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any



combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337- 11341(1992) (said references incorporated by reference in their entireties).

As discussed, supra, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of SEQ ID NO:Y may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NO:Y may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., Nature 331:84-86 (1988). The polypeptides of the present invention fused or conjugated to an antibody having disulfide- linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A 232,262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to



identify antagonists of hIL-5. (See, Bennett et al., J. Molecular Recognition 8:52-58 (1995); Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).

Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred  
 5 embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags  
 10 useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used  
 15 diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent  
 20 materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No.  
 25 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone,  
 30 fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin,



and aequorin; and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{111}\text{In}$  or  $^{99}\text{Tc}$ .

Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example,  $^{213}\text{Bi}$ . A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-

dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-

dichlorodiamine platinum (II) (DDP cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor,  $\alpha$ -interferon,  $\beta$ -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF- $\alpha$ , TNF- $\beta$ , AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, *Int. Immunol.*, 6:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"),



granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

### *Immunophenotyping*

The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific



epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al.*, *Cell*, 96:737-49 (1999)).

These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

#### *Assays For Antibody Binding*

The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C, adding protein A



and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., <sup>32</sup>P or <sup>125</sup>I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes



the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

10           The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g.,  $^3\text{H}$  or  $^{125}\text{I}$ ) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g.,  $^3\text{H}$  or  $^{125}\text{I}$ ) in the presence of increasing amounts of an unlabeled second antibody.

### *Therapeutic Uses*

25           The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, any



one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention,



including fragments thereof. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M,  $5 \times 10^{-5}$  M,  $10^{-5}$  M,  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M,  $10^{-8}$  M,  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, and  $10^{-15}$  M.

### *Gene Therapy*

In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., Clinical Pharmacy 12:488-505 (1993); Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, TIBTECH 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other



desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989). In specific

5   embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid- carrying vectors, or

10   indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be

15   accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun;

20   Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target

25   cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT

30   Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination



(Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)).

In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., Biotherapy 6:291-302 (1994), which describes the use of a retroviral vector to deliver the *mdr1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., J. Clin. Invest. 93:644-651 (1994); Kiem et al., Blood 83:1467-1473 (1994); Salmons and Gunzberg, Human Gene Therapy 4:129-141 (1993); and Grossman and Wilson, Curr. Opin. in Genetics and Devel. 3:110-114 (1993).

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, Current Opinion in Genetics and Development 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., Human Gene Therapy 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., Science 252:431-434 (1991); Rosenfeld et al., Cell 68:143-155 (1992); Mastrangeli et al., J. Clin. Invest. 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., Gene Therapy 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.



Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., Proc. Soc. Exp. Biol. Med. 204:289-300 (1993); U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, Meth. Enzymol. 217:599-618 (1993); Cohen et al., Meth. Enzymol. 217:618-644 (1993); Cline, Pharmac. Ther. 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as Tlymphocytes, Blymphocytes, monocytes, macrophages,



neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

## 20 *Demonstration of Therapeutic or Prophylactic Activity*

The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.



The invention provides methods of treatment, inhibi

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment;



this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when  
 5 administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, *Science* 249:1527-1533 (1990); Treat et  
 10 al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer,  
 15 *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and  
 20 Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., *Macromol. Sci. Rev. Macromol. Chem.* 23:61 (1983); see also Levy et al., *Science* 228:190 (1985); During et al., *Ann. Neurol.* 25:351 (1989); Howard et al., *J.Neurosurg.* 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose  
 25 (see, e.g., Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

In a specific embodiment where the compound of the invention is a nucleic  
 30 acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by



use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see e.g., Joliot et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of



the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.



For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

### *Diagnosis and Imaging*

Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level,



whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and technetium (99Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods



including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce  
5 diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of <sup>99m</sup>Tc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of  
10 Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the  
15 labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

In an embodiment, monitoring of the disease or disorder is carried out by  
20 repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label  
25 used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

30 In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with



a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

### *Kits*

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may



also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound



recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

### **Fusion Proteins**

5           Any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target  
10 cellular locations based on trafficking signals, the polypeptides of the present invention can be used as targeting molecules once fused to other proteins.

          Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur  
15 through linker sequences.

          Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence during purification from the host cell  
20 or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

          Moreover, polypeptides of the present invention, including fragments, and  
25 specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgA, IgE, IgG, IgM) or portions thereof (CH1, CH2, CH3, and any combination thereof, including both entire domains and portions thereof), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins  
30 consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., Nature 331:84-86 (1988).)



Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995).) Polynucleotides comprising or alternatively consisting of  
 5 nucleic acids which encode these fusion proteins are also encompassed by the invention.

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a  
 10 fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for  
 15 example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).)

Moreover, the polypeptides of the present invention can be fused to marker  
 20 sequences, such as a peptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for  
 25 instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984).)

Thus, any of these above fusions can be engineered using the polynucleotides  
 30 or the polypeptides of the present invention.

### **Vectors, Host Cells, and Protein Production**



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Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A,



pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlsbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

- 10 Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986). It is specifically contemplated that the
- 15 polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

- A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.
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- Polypeptides of the present invention, and preferably the secreted form, can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-
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mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some

5 proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

In one embodiment, the yeast *Pichia pastoris* is used to express the polypeptide of the present invention in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A

10 main step in the methanol metabolization pathway is the oxidation of methanol to formaldehyde using O<sub>2</sub>. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O<sub>2</sub>. Consequently, in a growth medium depending on methanol as a main

15 carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. See, Ellis, S.B., *et al.*, *Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J., *et al.*, *Yeast* 5:167-77 (1989); Tschopp, J.F., *et al.*, *Nucl. Acids Res.* 15:3859-76 (1987).

20 Thus, a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1* regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

In one example, the plasmid vector pPIC9K is used to express DNA encoding

25 a polypeptide of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a protein of the invention by virtue of the strong *AOX1* promoter linked to the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal

30 peptide (i.e., leader) located upstream of a multiple cloning site.



Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with the polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination, resulting in the formation of a new transcription unit (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; U.S. Patent No. 5,733,761, issued March 31, 1998; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y., and Hunkapiller et al., *Nature*, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a



polypeptide sequence of the invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid,  $\alpha$ -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid,  $\gamma$ -Abu,  $\epsilon$ -Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

The invention encompasses polypeptides which are differentially modified during or after translation, *e.g.*, by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease,  $\text{NaBH}_4$ ; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, *e.g.*, N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of the polypeptides of the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent NO: 4,179,337). The chemical moieties for



derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo *et al.*, *Appl. Biochem. Biotechnol.* 56:59-72 (1996); Vorobjev *et al.*, *Nucleosides Nucleotides* 18:2745-2750 (1999); and Caliceti *et al.*, *Bioconjug. Chem.* 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik *et al.*, *Exp. Hematol.* 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may



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As indicated above, pegylation of the proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992); Francis *et al.*, *Intern. J. of Hematol.* 68:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride ( $\text{ClSO}_2\text{CH}_2\text{CF}_3$ ). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoroethane sulphonyl group.

Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention.

The number of polyethylene glycol moieties attached to each protein of the invention (*i.e.*, the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12,



15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992).

The polypeptides of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the polypeptides of the invention, their preparation, and compositions (preferably, *Therapeutics*) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides corresponding to the amino acid sequence of SEQ ID NO:Y or encoded by the cDNA contained in a deposited clone (including fragments, variants, splice variants, and fusion proteins, corresponding to these polypeptides as described herein). These homomers may contain polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a multimer containing polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (*e.g.*, containing polypeptides having identical or different amino acid sequences) or a homotrimer (*e.g.*, containing polypeptides having identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (*i.e.*, polypeptides of different proteins) in addition to the polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional



embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

5 Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence ( e.g., that recited in the sequence listing, or contained in the polypeptide encoded by a deposited clone). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a fusion protein of the invention.

In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in an Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another protein that is capable of forming covalently associated multimers, such as for example, osteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627



(hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Another method for preparing multimer polypeptides of the invention involves  
5 use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, (1988)), and have since been found in a variety of different  
10 proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide  
15 sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are  
20 those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

25 In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention containing Flag® polypeptide sequence. In a further embodiment, associations proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti-  
30 Flag® antibody.

The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers



Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely modified by the addition of cysteine or biotin to the C terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hydrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into



liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

### **Uses of the Polynucleotides**

5           Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

          The polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers,  
10       since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each polynucleotide of the present invention can be used as a chromosome marker.

          Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in SEQ ID NO:X. Primers can be  
15       selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the SEQ ID NO:X will yield an amplified fragment.

20           Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled  
25       flow-sorted chromosomes, preselection by hybridization to construct chromosome specific-cDNA libraries and computer mapping techniques (See, e.g., Shuler, Trends Biotechnol 16:456-459 (1998) which is hereby incorporated by reference in its entirety)..

          Precise chromosomal location of the polynucleotides can also be achieved  
30       using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see

DDEE "E54T996D



Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes).

The polynucleotides of the present invention would likewise be useful for radiation hybrid mapping, HAPPY mapping, and long range restriction mapping. For a review of these techniques and others known in the art, see, e.g., Dear, "Genome Mapping: A Practical Approach," IRL Press at Oxford University Press, London (1997); Aydin, J. Mol. Med. 77:691-694 (1999); Hacia et al., Mol. Psychiatry 3:483-492 (1998); Herrick et al., Chromosome Res. 7:409-423 (1999); Hamilton et al., Methods Cell Biol. 62:265-280 (2000); and/or Ott, J. Hered. 90:68-70 (1999) each of which is hereby incorporated by reference in its entirety.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library) .) Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in the polynucleotide and the corresponding gene between affected and unaffected individuals can be examined. First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.



Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using polynucleotides of the present invention. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

Thus, the invention also provides a diagnostic method useful during diagnosis of a disorder, involving measuring the expression level of polynucleotides of the present invention in cells or body fluid from an individual and comparing the measured gene expression level with a standard level of polynucleotide expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a disorder.

In still another embodiment, the invention includes a kit for analyzing samples for the presence of proliferative and/or cancerous polynucleotides derived from a test subject. In a general embodiment, the kit includes at least one polynucleotide probe containing a nucleotide sequence that will specifically hybridize with a polynucleotide of the present invention and a suitable container. In a specific embodiment, the kit includes two polynucleotide probes defining an internal region of the polynucleotide of the present invention, where each probe has one strand containing a 31' mer-end internal to the region. In a further embodiment, the probes may be useful as primers for polymerase chain reaction amplification.

Where a diagnosis of a disorder, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed polynucleotide of the present invention expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "measuring the expression level of polynucleotide of the present invention" is intended qualitatively or quantitatively measuring or estimating the level of the polypeptide of the present invention or the level of the mRNA encoding the polypeptide in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide level or mRNA level in the first biological sample is measured or



estimated and compared to a standard polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having a disorder. As will be appreciated in the art, once a standard polypeptide  
5 level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains the polypeptide of the present invention or mRNA. As indicated, biological samples  
10 include body fluids (such as semen, lymph, sera, plasma, urine, synovial fluid and spinal fluid) which contain the polypeptide of the present invention, and other tissue sources found to express the polypeptide of the present invention. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred  
15 source.

The method(s) provided above may preferably be applied in a diagnostic method and/or kits in which polynucleotides and/or polypeptides are attached to a solid support. In one exemplary method, the support may be a "gene chip" or a "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174.  
20 Further, such a gene chip with polynucleotides of the present invention attached may be used to identify polymorphisms between the polynucleotide sequences, with polynucleotides isolated from a test subject. The knowledge of such polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying disease loci for many disorders, including cancerous diseases and conditions. Such a  
25 method is described in US Patents 5,858,659 and 5,856,104. The US Patents referenced supra are hereby incorporated by reference in their entirety herein.

The present invention encompasses polynucleotides of the present invention that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or according to other methods known in the art. The use of PNAs would serve as the  
30 preferred form if the polynucleotides are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a polyamide type of DNA analog and the monomeric units for adenine, guanine,



thymine and cytosine are available commercially (Perceptive Biosystems). Certain components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not present in PNAs. As disclosed by P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, *Science* 254, 1497 (1991); and M. Egholm, O. Buchardt, L.Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, and P. E. Nielsen, *Nature* 365, 666 (1993), PNAs bind specifically and tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency conditions than DNA/DNA duplexes, making it easier to perform multiplex hybridization. Smaller probes can be used than with DNA due to the strong binding. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer lowers the melting point ( $T_{sub.m}$ ) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can be done at low ionic strengths and reduce possible interference by salt during the analysis.

The present invention is useful for detecting cancer in mammals. In particular the invention is useful during diagnosis of pathological cell proliferative neoplasias which include, but are not limited to: acute myelogenous leukemias including acute monocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute erythroleukemia, acute megakaryocytic leukemia, and acute undifferentiated leukemia, etc.; and chronic myelogenous leukemias including chronic myelomonocytic leukemia, chronic granulocytic leukemia, etc. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

Pathological cell proliferative diseases, disorders, and/or conditions are often associated with inappropriate activation of proto-oncogenes. (Germann, E. P. et al., "The Etiology of Acute Leukemia: Molecular Genetics and Viral Oncology," in *Neoplastic Diseases of the Blood*, Vol 1., Wiernik, P. H. et al. eds., 161-182 (1985)). Neoplasias are now believed to result from the qualitative alteration of a normal

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cellular gene product, or from the quantitative modification of gene expression by insertion into the chromosome of a viral sequence, by chromosomal translocation of a gene to a more actively transcribed region, or by some other mechanism. (Gelmann et al., supra) It is likely that mutated or altered expression of specific genes is involved in the pathogenesis of some leukemias, among other tissues and cell types. (Gelmann et al., supra) Indeed, the human counterparts of the oncogenes involved in some animal neoplasias have been amplified or translocated in some cases of human leukemia and carcinoma. (Gelmann et al., supra)

For example, c-myc expression is highly amplified in the non-lymphocytic leukemia cell line HL-60. When HL-60 cells are chemically induced to stop proliferation, the level of c-myc is found to be downregulated. (International Publication Number WO 91/15580) However, it has been shown that exposure of HL-60 cells to a DNA construct that is complementary to the 5' end of c-myc or c-myb blocks translation of the corresponding mRNAs which downregulates expression of the c-myc or c-myb proteins and causes arrest of cell proliferation and differentiation of the treated cells. (International Publication Number WO 91/15580; Wickstrom et al., Proc. Natl. Acad. Sci. 85:1028 (1988); Anfossi et al., Proc. Natl. Acad. Sci. 86:3379 (1989)). However, the skilled artisan would appreciate the present invention's usefulness would not be limited to treatment of proliferative diseases, disorders, and/or conditions of hematopoietic cells and tissues, in light of the numerous cells and cell types of varying origins which are known to exhibit proliferative phenotypes.

In addition to the foregoing, a polynucleotide can be used to control gene expression through triple helix formation or antisense DNA or RNA. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). Both methods rely on binding of the polynucleotide to a complementary DNA or RNA. For these techniques, preferred polynucleotides are usually oligonucleotides 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456



(1988); and Dervan et al., Science 251:1360 (1991) ) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).) Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while  
 5 antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat or prevent disease.

Polynucleotides of the present invention are also useful in gene therapy. One  
 10 goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

15 The polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for  
 20 identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

The polynucleotides of the present invention can also be used as an alternative  
 25 to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA sequences. Once an unique ID database is established for an  
 30 individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.



Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, synovial fluid, amniotic fluid, breast milk, lymph, pulmonary sputum or surfactant, urine, fecal matter, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals. (Erich, H., PCR Technology, Freeman and Co. (1992).) Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, polynucleotides of the present invention can be used as polymorphic markers for forensic purposes.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to particular tissue prepared from the sequences of the present invention. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

### **Uses of the Polypeptides**

Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

A polypeptide of the present invention can be used to assay protein levels in a biological sample using antibody-based techniques. For example, protein expression



in tissues can be studied with classical immunohistological methods. (Jalkanen, M., et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., J. Cell . Biol. 105:3087-3096 (1987).) Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine ( $^{125}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{112}\text{In}$ ), and technetium ( $^{99\text{m}}\text{Tc}$ ), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying secreted protein levels in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example,  $^{131}\text{I}$ ,  $^{112}\text{In}$ ,  $^{99\text{m}}\text{Tc}$ ), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99\text{m}}\text{Tc}$ . The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).)



Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression of a polypeptide of the present invention in cells or body fluid of an individual; (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Moreover, polypeptides of the present invention can be used to treat, prevent, and/or diagnose disease. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B, SOD, catalase, DNA repair proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor suppressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth inhibition, enhancement of the immune response to proliferative cells or tissues).

Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat, prevent, and/or diagnose disease. For example, administration of an antibody directed to a polypeptide of the present invention can bind and reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also



be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

5

### **Gene Therapy Methods**

Another aspect of the present invention is to gene therapy methods for treating or preventing disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of a polypeptide of the present invention. This method requires a polynucleotide which codes for a polypeptide of the invention that operatively linked to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a polynucleotide of the invention *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, see Beldegrun et al., J. Natl. Cancer Inst., 85:207-216 (1993); Ferrantini et al., Cancer Research, 53:107-1112 (1993); Ferrantini et al., J. Immunology 153: 4604-4615 (1994); Kaido, T., et al., Int. J. Cancer 60: 221-229 (1995); Ogura et al., Cancer Research 50: 5102-5106 (1990); Santodonato, et al., Human Gene Therapy 7:1-10 (1996); Santodonato, et al., Gene Therapy 4:1246-1255 (1997); and Zhang, et al., Cancer Gene Therapy 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells which are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver,



and the like). The polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

In one embodiment, the polynucleotide of the invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences  
 5 that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such  
 10 methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

The polynucleotide vector constructs of the invention used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors  
 15 include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

Any strong promoter known to those skilled in the art can be used for driving  
 20 the expression of polynucleotide sequence of the invention. Suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter;  
 25 the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the b-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the polynucleotides of the invention.

Unlike other gene therapy techniques, one major advantage of introducing  
 30 naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA



sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct of the invention can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular, fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked *nucleic acid* sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.



The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

5       The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

10       In certain embodiments, the polynucleotide constructs of the invention are complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA ,  
15       84:7413-7416 (1987), which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA , 86:6077-6081 (1989), which is herein incorporated by reference); and purified transcription factors (Debs et al., J. Biol. Chem., 265:10189-10192 (1990), which is herein incorporated by reference), in functional form.

20       Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl Acad. Sci. USA , 84:7413-7416 (1987), which is herein incorporated by reference). Other commercially  
25       available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication NO: WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of  
30       DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., Felgner et al., Proc.



Natl. Acad. Sci. USA, 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

For example, commercially dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The various liposome-nucleic acid complexes are prepared using methods well known in the art. See, e.g., Straubinger et al., *Methods of Immunology*, 101:512-527 (1983), which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated. SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a



suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and then the preformed liposomes are mixed directly with the DNA. The liposome and

5 DNA form a very stable complex due to binding of the positively charged liposomes to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include  $\text{Ca}^{2+}$ -EDTA chelation (Papahadjopoulos et al., Biochim. Biophys. Acta, 394:483 (1975); Wilson et al., Cell, 17:77 (1979)); ether injection (Deamer et al.,

10 Biochim. Biophys. Acta, 443:629 (1976); Ostro et al., Biochem. Biophys. Res. Commun., 76:836 (1977); Fraley et al., Proc. Natl. Acad. Sci. USA, 76:3348 (1979)); detergent dialysis (Enoch et al., Proc. Natl. Acad. Sci. USA, 76:145 (1979)); and reverse-phase evaporation (REV) (Fraley et al., J. Biol. Chem., 255:10431 (1980); Szoka et al., Proc. Natl. Acad. Sci. USA, 75:145 (1978); Schaefer-Ridder et al.,

15 Science, 215:166 (1982)), which are herein incorporated by reference.

Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ration will be from about 5:1 to about 1:5. More preferably, the ration will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

U.S. Patent NO: 5,676,954 (which is herein incorporated by reference) reports

20 on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622,

25 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide methods for delivering DNA-cationic lipid complexes to mammals.

In certain embodiments, cells are engineered, *ex vivo* or *in vivo*, using a retroviral particle containing RNA which comprises a sequence encoding

30 polypeptides of the invention. Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis



virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected  
 5 include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy , 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation,  
 10 the use of liposomes, and CaPO<sub>4</sub> precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles which include polynucleotide encoding polypeptides of the invention. Such retroviral vector  
 15 particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express polypeptides of the invention.

In certain other embodiments, cells are engineered, *ex vivo* or *in vivo*, with polynucleotides of the invention contained in an adenovirus vector. Adenovirus can be manipulated such that it encodes and expresses polypeptides of the invention, and  
 20 at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartz et al., Am. Rev. Respir. Dis.,  
 25 109:233-238 (1974)). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld et al., Science , 252:431-434 (1991); Rosenfeld et al., Cell, 68:143-155 (1992)). Furthermore, extensive studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative  
 30 (Green et al. Proc. Natl. Acad. Sci. USA , 76:6606 (1979)).

Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, Curr. Opin. Genet. Devel., 3:499-503 (1993);



Rosenfeld et al., Cell , 68:143-155 (1992); Engelhardt et al., Human Genet. Ther., 4:759-769 (1993); Yang et al., Nature Genet., 7:362-369 (1994); Wilson et al., Nature , 365:691-692 (1993); and U.S. Patent NO: 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can  
 5 be grown in human 293 cells. These cells contain the E1 region of adenovirus and constitutively express E1a and E1b, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to Ad2, other varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

10 Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses  
 15 may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

In certain other embodiments, the cells are engineered, *ex vivo* or *in vivo*, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, Curr.  
 20 Topics in Microbiol. Immunol., 158:97 (1992)). It is also one of the few viruses that may integrate its DNA into non-dividing cells. Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678,  
 25 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

For example, an appropriate AAV vector for use in the present invention will include all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The polynucleotide construct containing polynucleotides of the invention is inserted into the AAV vector using standard cloning methods, such as those found  
 30 in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989). The recombinant AAV vector is then transfected into packaging cells which are infected with a helper virus, using any standard technique, including



lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses, cytomegaloviruses, vaccinia viruses, or herpes viruses. Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles which contain the polynucleotide construct of the invention.

5 These viral particles are then used to transduce eukaryotic cells, either *ex vivo* or *in vivo*. The transduced cells will contain the polynucleotide construct integrated into its genome, and will express the desired gene product.

Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding the polypeptide sequence of interest) via homologous recombination (see, e.g., U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA, 86:8932-8935 (1989); and Zijlstra et al., Nature, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made, using standard techniques known in the art, which contain the promoter with targeting sequences flanking the promoter. Suitable promoters are described herein. The targeting sequence is sufficiently complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5' end of the desired endogenous polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

25 The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.



The promoter-targeting sequence construct is delivered to the cells, either as naked polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can  
 5 be delivered by any method, included direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such  
 10 that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.

The polynucleotides encoding polypeptides of the present invention may be administered along with other polynucleotides encoding other angiogenic proteins. Angiogenic proteins include, but are not limited to, acidic and basic fibroblast growth  
 15 factors, VEGF-1, VEGF-2 (VEGF-C), VEGF-3 (VEGF-B), epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide synthase.

Preferably, the polynucleotide encoding a polypeptide of the invention  
 20 contains a secretory signal sequence that facilitates secretion of the protein. Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5' end of the coding region. The signal sequence may be homologous or heterologous to the polynucleotide of interest and may be  
 25 homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.

Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct  
 30 needle injection, systemic injection, catheter infusion, biolistic injectors, particle accelerators (i.e., "gene guns"), gelfoam sponge depots, other commercially available depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppositorial solid



(tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. For example, direct injection of naked calcium phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the  
5 rat livers. (Kaneda et al., Science, 243:375 (1989)).

A preferred method of local administration is by direct injection. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of arteries. Administration of a composition locally within the area of arteries refers to injecting  
10 the composition centimeters and preferably, millimeters within arteries.

Another method of local administration is to contact a polynucleotide construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue  
15 inside the wound.

Therapeutic compositions useful in systemic administration, include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a  
20 particular site.

Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad.  
25 Sci. USA , 189:11277-11281 (1992), which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a  
30 polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.



Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise condition requiring treatment and its severity, and the route of administration. The frequency of treatments depends upon a number of factors, such as the amount of polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian. Therapeutic compositions of the present invention can be administered to any animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly

### **Biological Activities**

The polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used in assays to test for one or more biological activities. If these polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological activity. Thus, the polynucleotides or polypeptides, or agonists or antagonists could be used to treat the associated disease.

### **Immune Activity**

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing diseases, disorders, and/or conditions of the immune system, by, for example, activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune diseases, disorders, and/or conditions may be genetic, somatic, such as cancer and some autoimmune diseases, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of



the present invention can be used as a marker or detector of a particular immune system disease or disorder.

Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing diseases, disorders, and/or conditions of hematopoietic cells. Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with a decrease in certain (or many) types hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein diseases, disorders, and/or conditions (e.g., agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, Digeorge Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

Moreover, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, polynucleotides or polypeptides, and/or agonists or antagonists of the present invention could be used to treat or prevent blood coagulation diseases, disorders, and/or conditions (e.g., afibrinogenemia, factor deficiencies), blood platelet diseases, disorders, and/or conditions (e.g., thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be important in the treatment or prevention of heart attacks (infarction), strokes, or scarring.

The polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing autoimmune disorders. Many autoimmune disorders result from inappropriate



recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of polynucleotides and polypeptides of the invention that can inhibit an immune response, particularly the proliferation, differentiation, or  
 5 chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

Autoimmune diseases or disorders that may be treated, prevented, and/or diagnosed by polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention include, but are not limited to, one or more of the following:  
 10 autoimmune hemolytic anemia, autoimmune neonatal thrombocytopenia, idiopathic thrombocytopenia purpura, autoimmunocytopenia, hemolytic anemia, antiphospholipid syndrome, dermatitis, allergic encephalomyelitis, myocarditis, relapsing polychondritis, rheumatic heart disease, glomerulonephritis (e.g. IgA nephropathy), Multiple Sclerosis, Neuritis, Uveitis Ophthalmia,  
 15 Polyendocrinopathies, Purpura (e.g., Henloch-Scoenlein purpura), Reiter's Disease, Stiff-Man Syndrome, Autoimmune Pulmonary Inflammation, Autism, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye, autoimmune thyroiditis, hypothyroidism (i.e., Hashimoto's thyroiditis, systemic lupus erythematosus, Goodpasture's syndrome, Pemphigus, Receptor autoimmunities such  
 20 as, for example, (a) Graves' Disease, (b) Myasthenia Gravis, and (c) insulin resistance, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, rheumatoid arthritis, scleroderma with anti-collagen antibodies, mixed connective tissue disease, polymyositis/dermatomyositis, pernicious anemia, idiopathic Addison's disease, infertility, glomerulonephritis such as primary glomerulonephritis and IgA  
 25 nephropathy, bullous pemphigoid, Sjogren's syndrome, diabetes mellitus, and adrenergic drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis), chronic active hepatitis, primary biliary cirrhosis, other endocrine gland failure, vitiligo, vasculitis, post-MI, cardiomyopathy syndrome, urticaria, atopic dermatitis, asthma, inflammatory myopathies, and other inflammatory, granulomatous,  
 30 degenerative, and atrophic disorders.

Additional autoimmune disorders (that are probable) that may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are



not limited to, rheumatoid arthritis (often characterized, e.g., by immune complexes in joints), scleroderma with anti-collagen antibodies (often characterized, e.g., by nucleolar and other nuclear antibodies), mixed connective tissue disease (often characterized, e.g., by antibodies to extractable nuclear antigens (e.g., ribonucleoprotein)), polymyositis (often characterized, e.g., by nonhistone ANA), pernicious anemia (often characterized, e.g., by antiparietal cell, microsomes, and intrinsic factor antibodies), idiopathic Addison's disease (often characterized, e.g., by humoral and cell-mediated adrenal cytotoxicity, infertility (often characterized, e.g., by antispermatzoal antibodies), glomerulonephritis (often characterized, e.g., by glomerular basement membrane antibodies or immune complexes), bullous pemphigoid (often characterized, e.g., by IgG and complement in basement membrane), Sjogren's syndrome (often characterized, e.g., by multiple tissue antibodies, and/or a specific nonhistone ANA (SS-B)), diabetes mellitus (often characterized, e.g., by cell-mediated and humoral islet cell antibodies), and adrenergic drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis) (often characterized, e.g., by beta-adrenergic receptor antibodies).

Additional autoimmune disorders (that are possible) that may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to, chronic active hepatitis (often characterized, e.g., by smooth muscle antibodies), primary biliary cirrhosis (often characterized, e.g., by mitochondrial antibodies), other endocrine gland failure (often characterized, e.g., by specific tissue antibodies in some cases), vitiligo (often characterized, e.g., by melanocyte antibodies), vasculitis (often characterized, e.g., by Ig and complement in vessel walls and/or low serum complement), post-MI (often characterized, e.g., by myocardial antibodies), cardiomyopathy syndrome (often characterized, e.g., by myocardial antibodies), urticaria (often characterized, e.g., by IgG and IgM antibodies to IgE), atopic dermatitis (often characterized, e.g., by IgG and IgM antibodies to IgE), asthma (often characterized, e.g., by IgG and IgM antibodies to IgE), and many other inflammatory, granulomatous, degenerative, and atrophic disorders.

In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated,



prevented, and/or diagnosed using for example, antagonists or agonists, polypeptides or polynucleotides, or antibodies of the present invention.

In a preferred embodiment polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention could be used as an agent to boost  
5 immunoresponsiveness among B cell and/or T cell immunodeficient individuals.

B cell immunodeficiencies that may be ameliorated or treated by administering the polypeptides or polynucleotides of the invention, and/or agonists thereof, include, but are not limited to, severe combined immunodeficiency (SCID)-X linked, SCID-autosomal, adenosine deaminase deficiency (ADA deficiency), X-  
10 linked agammaglobulinemia (XLA), Bruton's disease, congenital agammaglobulinemia, X-linked infantile agammaglobulinemia, acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, transient hypogammaglobulinemia of infancy, unspecified hypogammaglobulinemia,  
15 agammaglobulinemia, common variable immunodeficiency (CVI) (acquired), Wiskott-Aldrich Syndrome (WAS), X-linked immunodeficiency with hyper IgM, non X-linked immunodeficiency with hyper IgM, selective IgA deficiency, IgG subclass deficiency (with or without IgA deficiency), antibody deficiency with normal or elevated Igs, immunodeficiency with thymoma, Ig heavy chain deletions, kappa  
20 chain deficiency, B cell lymphoproliferative disorder (BLPD), selective IgM immunodeficiency, recessive agammaglobulinemia (Swiss type), reticular dysgenesis, neonatal neutropenia, severe congenital leukopenia, thymic aplasia or dysplasia with immunodeficiency, ataxia-telangiectasia, short limbed dwarfism, X-linked lymphoproliferative syndrome (XLP), Nezelof  
25 syndrome-combined immunodeficiency with Igs, purine nucleoside phosphorylase deficiency (PNP), MHC Class II deficiency (Bare Lymphocyte Syndrome) and severe combined immunodeficiency.

T cell deficiencies that may be ameliorated or treated by administering the polypeptides or polynucleotides of the invention, and/or agonists thereof include, but  
30 are not limited to, for example, DiGeorge anomaly, thymic hypoplasia, third and fourth pharyngeal pouch syndrome, 22q11.2 deletion, chronic mucocutaneous candidiasis, natural killer cell deficiency (NK), idiopathic CD4+ T-lymphocytopenia,



immunodeficiency with predominant T cell defect (unspecified), and unspecified immunodeficiency of cell mediated immunity. In specific embodiments, DiGeorge anomaly or conditions associated with DiGeorge anomaly are ameliorated or treated by, for example, administering the polypeptides or polynucleotides of the invention, or antagonists or agonists thereof.

Other immunodeficiencies that may be ameliorated or treated by administering polypeptides or polynucleotides of the invention, and/or agonists thereof, include, but are not limited to, severe combined immunodeficiency (SCID; e.g., X-linked SCID, autosomal SCID, and adenosine deaminase deficiency), ataxia-telangiectasia, Wiskott-Aldrich syndrome, short-limber dwarfism, X-linked lymphoproliferative syndrome (XLP), Nezelof syndrome (e.g., purine nucleoside phosphorylase deficiency), MHC Class II deficiency. In specific embodiments, ataxia-telangiectasia or conditions associated with ataxia-telangiectasia are ameliorated or treated by administering the polypeptides or polynucleotides of the invention, and/or agonists thereof.

In a specific preferred embodiment, rheumatoid arthritis is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In another specific preferred embodiment, systemic lupus erythematosus is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In another specific preferred embodiment, idiopathic thrombocytopenia purpura is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In another specific preferred embodiment IgA nephropathy is treated, prevented, and/or diagnosed using polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, and/or diagnosed using antibodies against the protein of the invention.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated, prevented, and/or diagnosed using polypeptides, antibodies, or polynucleotides of the invention, and/or



agonists or antagonists thereof. Moreover, these molecules can be used to treat, prevent, and/or diagnose anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

Moreover, inflammatory conditions may also be treated, diagnosed, and/or prevented with polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention. Such inflammatory conditions include, but are not limited to, for example, respiratory disorders (such as, e.g., asthma and allergy); gastrointestinal disorders (such as, e.g., inflammatory bowel disease); cancers (such as, e.g., gastric, ovarian, lung, bladder, liver, and breast); CNS disorders (such as, e.g., multiple sclerosis, blood-brain barrier permeability, ischemic brain injury and/or stroke, traumatic brain injury, neurodegenerative disorders (such as, e.g., Parkinson's disease and Alzheimer's disease), AIDS-related dementia, and prion disease); cardiovascular disorders (such as, e.g., atherosclerosis, myocarditis, cardiovascular disease, and cardiopulmonary bypass complications); as well as many additional diseases, conditions, and disorders that are characterized by inflammation (such as, e.g., chronic hepatitis (B and C), rheumatoid arthritis, gout, trauma, septic shock, pancreatitis, sarcoidosis, dermatitis, renal ischemia-reperfusion injury, Grave's disease, systemic lupus erythematosus, diabetes mellitus (i.e., type 1 diabetes), and allogenic transplant rejection).

In specific embodiments, polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, are useful to treat, diagnose, and/or prevent transplantation rejections, graft-versus-host disease, autoimmune and inflammatory diseases (e.g., immune complex-induced vasculitis, glomerulonephritis, hemolytic anemia, myasthenia gravis, type II collagen-induced arthritis, experimental allergic and hyperacute xenograft rejection, rheumatoid arthritis, and systemic lupus erythematosus (SLE)). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. Polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, that inhibit an immune response, particularly the activation, proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.



Similarly, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may also be used to modulate and/or diagnose inflammation. For example, since polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists of the invention may inhibit the activation, proliferation and/or differentiation of cells involved in an inflammatory response, these molecules can be used to treat, diagnose, or prognose, inflammatory conditions, both chronic and acute conditions, including, but not limited to, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, and resulting from over production of cytokines (e.g., TNF or IL-1.).

Polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the invention can be used to treat, detect, and/or prevent infectious agents. For example, by increasing the immune response, particularly increasing the proliferation activation and/or differentiation of B and/or T cells, infectious diseases may be treated, detected, and/or prevented. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may also directly inhibit the infectious agent (refer to section of application listing infectious agents, etc), without necessarily eliciting an immune response.

Additional preferred embodiments of the invention include, but are not limited to, the use of polypeptides, antibodies, polynucleotides and/or agonists or antagonists in the following applications:

Administration to an animal (e.g., mouse, rat, rabbit, hamster, guinea pig, pigs, micro-pig, chicken, camel, goat, horse, cow, sheep, dog, cat, non-human primate, and human, most preferably human) to boost the immune system to produce increased quantities of one or more antibodies (e.g., IgG, IgA, IgM, and IgE), to induce higher affinity antibody production (e.g., IgG, IgA, IgM, and IgE), and/or to increase an immune response.



Administration to an animal (including, but not limited to, those listed above, and also including transgenic animals) incapable of producing functional endogenous antibody molecules or having an otherwise compromised endogenous immune system, but which is capable of producing human immunoglobulin molecules by means of a reconstituted or partially reconstituted immune system from another animal (see, e.g., published PCT Application Nos. WO98/24893, WO/9634096, WO/9633735, and WO/9110741.

A vaccine adjuvant that enhances immune responsiveness to specific antigen.

An adjuvant to enhance tumor-specific immune responses.

- 10 An adjuvant to enhance anti-viral immune responses. Anti-viral immune responses that may be enhanced using the compositions of the invention as an adjuvant, include virus and virus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: AIDS, meningitis, Dengue, EBV, and hepatitis (e.g., hepatitis B). In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: HIV/AIDS, Respiratory syncytial virus, Dengue, Rotavirus, Japanese B encephalitis, Influenza A and B, Parainfluenza, Measles, Cytomegalovirus, Rabies, Junin, Chikungunya, Rift Valley fever, Herpes simplex, and yellow fever.

- 25 An adjuvant to enhance anti-bacterial or anti-fungal immune responses. Anti-bacterial or anti-fungal immune responses that may be enhanced using the compositions of the invention as an adjuvant, include bacteria or fungus and bacteria or fungus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: tetanus, Diphtheria, botulism, and meningitis type B. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: *Vibrio cholerae*, *Mycobacterium leprae*, *Salmonella typhi*, *Salmonella paratyphi*, *Meisseria meningitidis*,



*Streptococcus pneumoniae*, Group B streptococcus, *Shigella spp.*, Enterotoxigenic *Escherichia coli*, Enterohemorrhagic *E. coli*, *Borrelia burgdorferi*, and Plasmodium (malaria).

An adjuvant to enhance anti-parasitic immune responses. Anti-parasitic  
 5 immune responses that may be enhanced using the compositions of the invention as an adjuvant, include parasite and parasite associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a parasite. In another specific embodiment, the compositions of the invention are used as an  
 10 adjuvant to enhance an immune response to Plasmodium (malaria).

As a stimulator of B cell responsiveness to pathogens.

As an activator of T cells.

As an agent that elevates the immune status of an individual prior to their receipt of immunosuppressive therapies.

15 As an agent to induce higher affinity antibodies.

As an agent to increase serum immunoglobulin concentrations.

As an agent to accelerate recovery of immunocompromised individuals.

As an agent to boost immunoresponsiveness among aged populations.

As an immune system enhancer prior to, during, or after bone marrow  
 20 transplant and/or other transplants (e.g., allogeneic or xenogeneic organ transplantation). With respect to transplantation, compositions of the invention may be administered prior to, concomitant with, and/or after transplantation. In a specific embodiment, compositions of the invention are administered after transplantation, prior to the beginning of recovery of T-cell populations. In another specific  
 25 embodiment, compositions of the invention are first administered after transplantation after the beginning of recovery of T cell populations, but prior to full recovery of B cell populations.

As an agent to boost immunoresponsiveness among individuals having an acquired loss of B cell function. Conditions resulting in an acquired loss of B cell  
 30 function that may be ameliorated or treated by administering the polypeptides, antibodies, polynucleotides and/or agonists or antagonists thereof, include, but are







As pretreatment of bone marrow samples prior to transplant. Such treatment would increase B cell representation and thus accelerate recover.

Additionally, polypeptides or polynucleotides of the invention, and/or agonists thereof, may be used to treat or prevent IgE-mediated allergic reactions. Such allergic reactions include, but are not limited to, asthma, rhinitis, and eczema.

Antagonists of the invention include, for example, binding and/or inhibitory antibodies, antisense nucleic acids, or ribozymes. These would be expected to reverse many of the activities of the ligand described above as well as find clinical or practical application as:

A therapy for preventing the B cell proliferation and Ig secretion associated with autoimmune diseases such as idiopathic thrombocytopenic purpura, systemic lupus erythramatosus and MS.

An inhibitor of graft versus host disease or transplant rejection.

30           A therapy for chronic hypergammaglobulinemia evident in such diseases as  
monoclonal gammopathy of undetermined significance (MGUS), Waldenström's  
disease, related idiopathic monoclonal gammopathies, and plasmacytomas.



A therapy for decreasing cellular proliferation of Large B-cell Lymphomas.

A means of decreasing the involvement of B cells and Ig associated with Chronic Myelogenous Leukemia.

An immunosuppressive agent(s).

- 5 Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to modulate IgE concentrations in vitro or in vivo.

In another embodiment, administration of polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the invention, may be used to treat or prevent IgE-mediated allergic reactions including, but not limited to, asthma, rhinitis, and eczema.

10 The agonists and antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described herein.

The agonists or antagonists may be employed for instance to inhibit polypeptide chemotaxis and activation of macrophages and their precursors, and of neutrophils, basophils, B lymphocytes and some T-cell subsets, e.g., activated and CD8 cytotoxic T cells and natural killer cells, in certain auto-immune and chronic inflammatory and infective diseases. Examples of autoimmune diseases are described herein and include multiple sclerosis, and insulin-dependent diabetes. The antagonists or agonists may also be employed to treat infectious diseases including silicosis, sarcoidosis, idiopathic pulmonary fibrosis by, for example, preventing the recruitment and activation of mononuclear phagocytes. They may also be employed to treat idiopathic hyper-eosinophilic syndrome by, for example, preventing eosinophil production and migration. The antagonists or agonists or may also be employed for treating atherosclerosis, for example, by preventing monocyte infiltration in the artery wall.

25 Antibodies against polypeptides of the invention may be employed to treat ARDS.

Agonists and/or antagonists of the invention also have uses in stimulating wound and tissue repair, stimulating angiogenesis, stimulating the repair of vascular or lymphatic diseases or disorders. Additionally, agonists and antagonists of the invention may be used to stimulate the regeneration of mucosal surfaces.

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In a specific embodiment, polynucleotides or polypeptides, and/or agonists thereof are used to treat or prevent a disorder characterized by primary or acquired immunodeficiency, deficient serum immunoglobulin production, recurrent infections, and/or immune system dysfunction. Moreover, polynucleotides or polypeptides, and/or agonists thereof may be used to treat or prevent infections of the joints, bones, skin, and/or parotid glands, blood-borne infections (e.g., sepsis, meningitis, septic arthritis, and/or osteomyelitis), autoimmune diseases (e.g., those disclosed herein), inflammatory disorders, and malignancies, and/or any disease or disorder or condition associated with these infections, diseases, disorders and/or malignancies) including, but not limited to, CVID, other primary immune deficiencies, HIV disease, CLL, recurrent bronchitis, sinusitis, otitis media, conjunctivitis, pneumonia, hepatitis, meningitis, herpes zoster (e.g., severe herpes zoster), and/or pneumocystis carinii.

In another embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention are used to treat, and/or diagnose an individual having common variable immunodeficiency disease ("CVID"; also known as "acquired agammaglobulinemia" and "acquired hypogammaglobulinemia") or a subset of this disease.

In a specific embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be used to treat, diagnose, and/or prevent (1) cancers or neoplasms and (2) autoimmune cell or tissue-related cancers or neoplasms. In a preferred embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention conjugated to a toxin or a radioactive isotope, as described herein, may be used to treat, diagnose, and/or prevent acute myelogenous leukemia. In a further preferred embodiment, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention conjugated to a toxin or a radioactive isotope, as described herein, may be used to treat, diagnose, and/or prevent, chronic myelogenous leukemia, multiple myeloma, non-Hodgkins lymphoma, and/or Hodgkins disease.

In another specific embodiment, polynucleotides or polypeptides, and/or agonists or antagonists of the invention may be used to treat, diagnose, prognose, and/or prevent selective IgA deficiency, myeloperoxidase deficiency, C2 deficiency, ataxia-telangiectasia, DiGeorge anomaly, common variable immunodeficiency (CVI),



X-linked agammaglobulinemia, severe combined immunodeficiency (SCID), chronic granulomatous disease (CGD), and Wiskott-Aldrich syndrome.

Examples of autoimmune disorders that can be treated or detected are described above and also include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitis, and autoimmune inflammatory eye disease.

In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prognosed, prevented, and/or diagnosed using antibodies against the polypeptide of the invention.

As an agent to boost immunoresponsiveness among B cell immunodeficient individuals, such as, for example, an individual who has undergone a partial or complete splenectomy.

Additionally, polynucleotides, polypeptides, and/or antagonists of the invention may affect apoptosis, and therefore, would be useful in treating a number of diseases associated with increased cell survival or the inhibition of apoptosis. For example, diseases associated with increased cell survival or the inhibition of apoptosis that could be treated or detected by polynucleotides, polypeptides, and/or antagonists of the invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related



glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. In preferred embodiments, polynucleotides, polypeptides, and/or antagonists of the invention are used to inhibit growth, progression, and/or metastasis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be treated or detected by polynucleotides, polypeptides, and/or antagonists of the invention, include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be treated or detected by polynucleotides, polypeptides, and/or antagonists of the invention, include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and



brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestosis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

Hyperproliferative diseases and/or disorders that could be detected and/or treated by polynucleotides, polypeptides, and/or antagonists of the invention, include, but are not limited to neoplasms located in the: liver, abdomen, bone, breast, digestive system, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

Similarly, other hyperproliferative disorders can also be treated or detected by polynucleotides, polypeptides, and/or antagonists of the invention. Examples of such hyperproliferative disorders include, but are not limited to:

hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenström's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

## **Hyperproliferative Disorders**

A polynucleotides or polypeptides, or agonists or antagonists of the invention can be used to treat, prevent, and/or diagnose hyperproliferative diseases, disorders, and/or conditions, including neoplasms. A polynucleotides or polypeptides, or agonists or antagonists of the present invention may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, a polynucleotides or polypeptides, or agonists or antagonists of the present invention may proliferate other cells which can inhibit the hyperproliferative disorder.



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preventing cell-proliferative diseases, disorders, and/or conditions in individuals



comprising administration of one or more active gene copies of the present invention to an abnormally proliferating cell or cells. In a preferred embodiment, polynucleotides of the present invention is a DNA construct comprising a recombinant expression vector effective in expressing a DNA sequence encoding said polynucleotides. In another preferred embodiment of the present invention, the DNA construct encoding the polynucleotides of the present invention is inserted into cells to be treated utilizing a retrovirus, or more preferably an adenoviral vector (See G J. Nabel, et. al., PNAS 1999 96: 324-326, which is hereby incorporated by reference). In a most preferred embodiment, the viral vector is defective and will not transform non-proliferating cells, only proliferating cells. Moreover, in a preferred embodiment, the polynucleotides of the present invention inserted into proliferating cells either alone, or in combination with or fused to other polynucleotides, can then be modulated via an external stimulus (i.e. magnetic, specific small molecule, chemical, or drug administration, etc.), which acts upon the promoter upstream of said polynucleotides to induce expression of the encoded protein product. As such the beneficial therapeutic affect of the present invention may be expressly modulated (i.e. to increase, decrease, or inhibit expression of the present invention) based upon said external stimulus.

Polynucleotides of the present invention may be useful in repressing expression of oncogenic genes or antigens. By "repressing expression of the oncogenic genes " is intended the suppression of the transcription of the gene, the degradation of the gene transcript (pre-message RNA), the inhibition of splicing, the destruction of the messenger RNA, the prevention of the post-translational modifications of the protein, the destruction of the protein, or the inhibition of the normal function of the protein.

For local administration to abnormally proliferating cells, polynucleotides of the present invention may be administered by any method known to those of skill in the art including, but not limited to transfection, electroporation, microinjection of cells, or in vehicles such as liposomes, lipofectin, or as naked polynucleotides, or any other method described throughout the specification. The polynucleotide of the present invention may be delivered by known gene delivery systems such as, but not limited to, retroviral vectors (Gilboa, J. Virology 44:845 (1982); Hocke, Nature



5 In order to specifically deliver or transfect cells which are abnormally proliferating and spare non-dividing cells, it is preferable to utilize a retrovirus, or adenoviral (as described in the art and elsewhere herein) delivery system known to those of skill in the art. Since host DNA replication is required for retroviral DNA to integrate and the retrovirus will be unable to self replicate due to the lack of the retrovirus genes  
10 needed for its life cycle. Utilizing such a retroviral delivery system for polynucleotides of the present invention will target said gene and constructs to abnormally proliferating cells and will spare the non-dividing normal cells.

By "cell proliferative disease" is meant any human or animal disease or disorder, affecting any one or any combination of organs, cavities, or body parts, which is characterized by single or multiple local abnormal proliferations of cells, groups of cells, or tissues, whether benign or malignant.

Any amount of the polynucleotides of the present invention may be administered as long as it has a biologically inhibiting effect on the proliferation of the treated cells. Moreover, it is possible to administer more than one of the polynucleotide of the present invention simultaneously to the same site. By "biologically inhibiting" is meant partial or total growth inhibition as well as decreases in the rate of proliferation or growth of the cells. The biologically inhibitory dose may be determined by assessing the effects of the polynucleotides of the present invention on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell cultures, or any other method known to one of ordinary skill in the art.



The present invention is further directed to antibody-based therapies which involve administering of anti-polypeptides and anti-polynucleotide antibodies to a mammalian, preferably human, patient for treating, preventing, and/or diagnosing one or more of the described diseases, disorders, and/or conditions. Methods for producing anti-polypeptides and anti-polynucleotide antibodies polyclonal and monoclonal antibodies are described in detail elsewhere herein. Such antibodies may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

In particular, the antibodies, fragments and derivatives of the present invention are useful for treating, preventing, and/or diagnosing a subject having or developing cell proliferative and/or differentiation diseases, disorders, and/or conditions as described herein. Such treatment comprises administering a single or multiple doses of the antibody, or a fragment, derivative, or a conjugate thereof.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors, for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of diseases, disorders, and/or conditions related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides, including fragments thereof. Preferred binding affinities include those



with a dissociation constant or  $K_d$  less than  $5 \times 10^{-6}M$ ,  $10^{-6}M$ ,  $5 \times 10^{-7}M$ ,  $10^{-7}M$ ,  $5 \times 10^{-8}M$ ,  $10^{-8}M$ ,  $5 \times 10^{-9}M$ ,  $10^{-9}M$ ,  $5 \times 10^{-10}M$ ,  $10^{-10}M$ ,  $5 \times 10^{-11}M$ ,  $10^{-11}M$ ,  $5 \times 10^{-12}M$ ,  $10^{-12}M$ ,  $5 \times 10^{-13}M$ ,  $10^{-13}M$ ,  $5 \times 10^{-14}M$ ,  $10^{-14}M$ ,  $5 \times 10^{-15}M$ , and  $10^{-15}M$ .

Moreover, polypeptides of the present invention are useful in inhibiting the  
 5 angiogenesis of proliferative cells or tissues, either alone, as a protein fusion, or in  
 combination with other polypeptides directly or indirectly, as described elsewhere  
 herein. In a most preferred embodiment, said anti-angiogenesis effect may be  
 achieved indirectly, for example, through the inhibition of hematopoietic, tumor-  
 specific cells, such as tumor-associated macrophages (See Joseph IB, et al. J Natl  
 10 Cancer Inst, 90(21):1648-53 (1998), which is hereby incorporated by reference).  
 Antibodies directed to polypeptides or polynucleotides of the present invention may  
 also result in inhibition of angiogenesis directly, or indirectly (See Witte L, et al.,  
 Cancer Metastasis Rev. 17(2):155-61 (1998), which is hereby incorporated by  
 reference)).

15 Polypeptides, including protein fusions, of the present invention, or fragments  
 thereof may be useful in inhibiting proliferative cells or tissues through the induction  
 of apoptosis. Said polypeptides may act either directly, or indirectly to induce  
 apoptosis of proliferative cells and tissues, for example in the activation of a death-  
 domain receptor, such as tumor necrosis factor (TNF) receptor-1, CD95 (Fas/APO-1),  
 20 TNF-receptor-related apoptosis-mediated protein (TRAMP) and TNF-related  
 apoptosis-inducing ligand (TRAIL) receptor-1 and -2 (See Schulze-Osthoff K, et.al.,  
 Eur J Biochem 254(3):439-59 (1998), which is hereby incorporated by reference).  
 Moreover, in another preferred embodiment of the present invention, said  
 polypeptides may induce apoptosis through other mechanisms, such as in the  
 25 activation of other proteins which will activate apoptosis, or through stimulating the  
 expression of said proteins, either alone or in combination with small molecule drugs  
 or adjuvants, such as apoptonin, galectins, thioredoxins, antiinflammatory proteins  
 (See for example, Mutat Res 400(1-2):447-55 (1998), Med Hypotheses.50(5):423-33  
 (1998), Chem Biol Interact. Apr 24;111-112:23-34 (1998), J Mol Med.76(6):402-12  
 30 (1998), Int J Tissue React;20(1):3-15 (1998), which are all hereby incorporated by  
 reference).



Polypeptides, including protein fusions to, or fragments thereof, of the present invention are useful in inhibiting the metastasis of proliferative cells or tissues. Inhibition may occur as a direct result of administering polypeptides, or antibodies directed to said polypeptides as described elsewhere herein, or indirectly, such as activating the expression of proteins known to inhibit metastasis, for example alpha 4 integrins, (See, e.g., Curr Top Microbiol Immunol 1998;231:125-41, which is hereby incorporated by reference). Such therapeutic affects of the present invention may be achieved either alone, or in combination with small molecule drugs or adjuvants.

In another embodiment, the invention provides a method of delivering compositions containing the polypeptides of the invention (e.g., compositions containing polypeptides or polypeptide antibodies associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs) to targeted cells expressing the polypeptide of the present invention. Polypeptides or polypeptide antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions.

Polypeptides, protein fusions to, or fragments thereof, of the present invention are useful in enhancing the immunogenicity and/or antigenicity of proliferating cells or tissues, either directly, such as would occur if the polypeptides of the present invention 'vaccinated' the immune response to respond to proliferative antigens and immunogens, or indirectly, such as in activating the expression of proteins known to enhance the immune response (e.g. chemokines), to said antigens and immunogens.

### **Cardiovascular Disorders**

Polynucleotides or polypeptides, or agonists or antagonists of the invention may be used to treat, prevent, and/or diagnose cardiovascular diseases, disorders, and/or conditions, including peripheral artery disease, such as limb ischemia.

Cardiovascular diseases, disorders, and/or conditions include cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary atresia, and Scimitar Syndrome. Congenital heart defects include aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia, patent ductus



arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome, levocardia, tetralogy of fallot, transposition of great vessels, double outlet right ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion defects, Lutembacher's Syndrome, trilog y of Fallot, ventricular heart septal defects.

Cardiovascular diseases, disorders, and/or conditions also include heart disease, such as arrhythmias, carcinoid heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right ventricular hypertrophy, post-infarction heart rupture, ventricular septal rupture, heart valve diseases, myocardial diseases, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous), pneumopericardium, postpericardiotomy syndrome, pulmonary heart disease, rheumatic heart disease, ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and cardiovascular tuberculosis.

Arrhythmias include sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extrasystole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial block, long QT syndrome, parasystole, Lown-Ganong-Levine Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus syndrome, tachycardias, and ventricular fibrillation. Tachycardias include paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal reentry tachycardia, ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia, Torsades de Pointes, and ventricular tachycardia.

Heart valve disease include aortic valve insufficiency, aortic valve stenosis, hear murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve insufficiency, pulmonary valve stenosis, tricuspid atresia, tricuspid valve insufficiency, and tricuspid valve stenosis.



Myocardial diseases include alcoholic cardiomyopathy, congestive cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular stenosis, pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Kearns Syndrome, myocardial reperfusion injury, and myocarditis.

Myocardial ischemias include coronary disease, such as angina pectoris, coronary aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasospasm, myocardial infarction and myocardial stunning.

Cardiovascular diseases also include vascular diseases such as aneurysms, angiodyplasia, angiomas, bacillary angiomas, Hippel-Lindau Disease, Klippel-Trenaunay-Weber Syndrome, Sturge-Weber Syndrome, angioneurotic edema, aortic diseases, Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive diseases, arteritis, enarteritis, polyarteritis nodosa, cerebrovascular diseases, disorders, and/or conditions, diabetic angiopathies, diabetic retinopathy, embolisms, thrombosis, erythromelalgia, hemorrhoids, hepatic veno-occlusive disease, hypertension, hypotension, ischemia, peripheral vascular diseases, phlebitis, pulmonary veno-occlusive disease, Raynaud's disease, CREST syndrome, retinal vein occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, ataxia telangiectasia, hereditary hemorrhagic telangiectasia, varicocele, varicose veins, varicose ulcer, vasculitis, and venous insufficiency.

Aneurysms include dissecting aneurysms, false aneurysms, infected aneurysms, ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart aneurysms, and iliac aneurysms.

Arterial occlusive diseases include arteriosclerosis, intermittent claudication, carotid stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease, renal artery obstruction, retinal artery occlusion, and thromboangiitis obliterans.

Cerebrovascular diseases, disorders, and/or conditions include carotid artery diseases, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformation, cerebral artery diseases, cerebral embolism and thrombosis, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural



hematoma, subarachnoid hemorrhage, cerebral infarction, cerebral ischemia (including transient), subclavian steal syndrome, periventricular leukomalacia, vascular headache, cluster headache, migraine, and vertebrobasilar insufficiency.

Embolisms include air embolisms, amniotic fluid embolisms, cholesterol  
5 embolisms, blue toe syndrome, fat embolisms, pulmonary embolisms, and  
thromboembolisms. Thrombosis include coronary thrombosis, hepatic vein  
thrombosis, retinal vein occlusion, carotid artery thrombosis, sinus thrombosis,  
Wallenberg's syndrome, and thrombophlebitis.

Ischemia includes cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion injuries, and peripheral limb ischemia. Vasculitis includes aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome, mucocutaneous lymph node syndrome, thromboangiitis obliterans, hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and Wegener's granulomatosis.

15 Polynucleotides or polypeptides, or agonists or antagonists of the invention, are especially effective for the treatment of critical limb ischemia and coronary disease.

Polypeptides may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, biolistic injectors, particle accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppository solid pharmaceutical formulations, decanting or topical applications during surgery, aerosol delivery. Such methods are known in the art. Polypeptides of the invention may be administered as part of a *Therapeutic*, described in more detail below. Methods of delivering polynucleotides of the invention are described in more detail herein.

### **Anti-Angiogenesis Activity**

The naturally occurring balance between endogenous stimulators and  
 30 inhibitors of angiogenesis is one in which inhibitory influences predominate.  
 Rastinejad *et al.*, *Cell* 56:345-355 (1989). In those rare instances in which  
 neovascularization occurs under normal physiological conditions, such as wound



healing, organ regeneration, embryonic development, and female reproductive processes, angiogenesis is stringently regulated and spatially and temporally delimited. Under conditions of pathological angiogenesis such as that characterizing solid tumor growth, these regulatory controls fail. Unregulated angiogenesis becomes pathologic and sustains progression of many neoplastic and non-neoplastic diseases. A number of serious diseases are dominated by abnormal neovascularization including solid tumor growth and metastases, arthritis, some types of eye diseases, disorders, and/or conditions, and psoriasis. See, e.g., reviews by Moses *et al.*, *Biotech.* 9:630-634 (1991); Folkman *et al.*, *N. Engl. J. Med.*, 333:1757-1763 (1995); Auerbach *et al.*, *J. Microvasc. Res.* 29:401-411 (1985); Folkman, *Advances in Cancer Research*, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, *Am. J. Ophthalmol.* 94:715-743 (1982); and Folkman *et al.*, *Science* 221:719-725 (1983). In a number of pathological conditions, the process of angiogenesis contributes to the disease state. For example, significant data have accumulated which suggest that the growth of solid tumors is dependent on angiogenesis. Folkman and Klagsbrun, *Science* 235:442-447 (1987).

The present invention provides for treatment of diseases, disorders, and/or conditions associated with neovascularization by administration of the polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists of the present invention. Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides, or agonists or antagonists of the invention include, but are not limited to, malignancies, solid tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman *et al.*, *Medicine*, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)). Thus, the present invention provides a method of treating, preventing, and/or diagnosing an angiogenesis-related disease and/or disorder, comprising administering to an individual in need thereof a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist of the invention. For example, polynucleotides, polypeptides, antagonists and/or agonists may be utilized in a variety of additional methods in order to therapeutically treat or prevent a cancer or tumor. Cancers which may be treated, prevented, and/or diagnosed with polynucleotides, polypeptides, antagonists and/or agonists include, but are not limited to solid tumors,



including prostate, lung, breast, ovarian, stomach, pancreas, larynx, esophagus, testes, liver, parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder, thyroid cancer; primary tumors and metastases; melanomas; glioblastoma; Kaposi's sarcoma; leiomyosarcoma; non- small cell lung cancer; colorectal cancer; advanced malignancies; and blood born tumors such as leukemias. For example, polynucleotides, polypeptides, antagonists and/or agonists may be delivered topically, in order to treat or prevent cancers such as skin cancer, head and neck tumors, breast tumors, and Kaposi's sarcoma.

Within yet other aspects, polynucleotides, polypeptides, antagonists and/or agonists may be utilized to treat superficial forms of bladder cancer by, for example, intravesical administration. Polynucleotides, polypeptides, antagonists and/or agonists may be delivered directly into the tumor, or near the tumor site, via injection or a catheter. Of course, as the artisan of ordinary skill will appreciate, the appropriate mode of administration will vary according to the cancer to be treated. Other modes of delivery are discussed herein.

Polynucleotides, polypeptides, antagonists and/or agonists may be useful in treating, preventing, and/or diagnosing other diseases, disorders, and/or conditions, besides cancers, which involve angiogenesis. These diseases, disorders, and/or conditions include, but are not limited to: benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; arteriosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uveitis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophilic joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis.

For example, within one aspect of the present invention methods are provided for treating, preventing, and/or diagnosing hypertrophic scars and keloids, comprising



the step of administering a polynucleotide, polypeptide, antagonist and/or agonist of the invention to a hypertrophic scar or keloid.

Within one embodiment of the present invention polynucleotides, polypeptides, antagonists and/or agonists are directly injected into a hypertrophic scar or keloid, in order to prevent the progression of these lesions. This therapy is of particular value in the prophylactic treatment of conditions which are known to result in the development of hypertrophic scars and keloids (e.g., burns), and is preferably initiated after the proliferative phase has had time to progress (approximately 14 days after the initial injury), but before hypertrophic scar or keloid development. As noted above, the present invention also provides methods for treating, preventing, and/or diagnosing neovascular diseases of the eye, including for example, corneal neovascularization, neovascular glaucoma, proliferative diabetic retinopathy, retrolental fibroplasia and macular degeneration.

Moreover, Ocular diseases, disorders, and/or conditions associated with neovascularization which can be treated, prevented, and/or diagnosed with the polynucleotides and polypeptides of the present invention (including agonists and/or antagonists) include, but are not limited to: neovascular glaucoma, diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity macular degeneration, corneal graft neovascularization, as well as other eye inflammatory diseases, ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.g., reviews by Waltman *et al.*, *Am. J. Ophthalmol.* 85:704-710 (1978) and Gartner *et al.*, *Surv. Ophthalmol.* 22:291-312 (1978).

Thus, within one aspect of the present invention methods are provided for treating or preventing neovascular diseases of the eye such as corneal neovascularization (including corneal graft neovascularization), comprising the step of administering to a patient a therapeutically effective amount of a compound (as described above) to the cornea, such that the formation of blood vessels is inhibited. Briefly, the cornea is a tissue which normally lacks blood vessels. In certain pathological conditions however, capillaries may extend into the cornea from the pericorneal vascular plexus of the limbus. When the cornea becomes vascularized, it also becomes clouded, resulting in a decline in the patient's visual acuity. Visual loss may become complete if the cornea completely opacitates. A wide variety of



diseases, disorders, and/or conditions can result in corneal neovascularization, including for example, corneal infections (e.g., trachoma, herpes simplex keratitis, leishmaniasis and onchocerciasis), immunological processes (e.g., graft rejection and Stevens-Johnson's syndrome), alkali burns, trauma, inflammation (of any cause),  
5 toxic and nutritional deficiency states, and as a complication of wearing contact lenses.

Within particularly preferred embodiments of the invention, may be prepared for topical administration in saline (combined with any of the preservatives and antimicrobial agents commonly used in ocular preparations), and administered in  
10 eyedrop form. The solution or suspension may be prepared in its pure form and administered several times daily. Alternatively, anti-angiogenic compositions, prepared as described above, may also be administered directly to the cornea. Within preferred embodiments, the anti-angiogenic composition is prepared with a muco-adhesive polymer which binds to cornea. Within further embodiments, the anti-  
15 angiogenic factors or anti-angiogenic compositions may be utilized as an adjunct to conventional steroid therapy. Topical therapy may also be useful prophylactically in corneal lesions which are known to have a high probability of inducing an angiogenic response (such as chemical burns). In these instances the treatment, likely in combination with steroids, may be instituted immediately to help prevent subsequent  
20 complications.

Within other embodiments, the compounds described above may be injected directly into the corneal stroma by an ophthalmologist under microscopic guidance. The preferred site of injection may vary with the morphology of the individual lesion, but the goal of the administration would be to place the composition at the advancing  
25 front of the vasculature (i.e., interspersed between the blood vessels and the normal cornea). In most cases this would involve perilimbic corneal injection to "protect" the cornea from the advancing blood vessels. This method may also be utilized shortly after a corneal insult in order to prophylactically prevent corneal neovascularization. In this situation the material could be injected in the perilimbic cornea interspersed  
30 between the corneal lesion and its undesired potential limbic blood supply. Such methods may also be utilized in a similar fashion to prevent capillary invasion of transplanted corneas. In a sustained-release form injections might only be required 2-



3 times per year. A steroid could also be added to the injection solution to reduce inflammation resulting from the injection itself.

Within another aspect of the present invention, methods are provided for treating or preventing neovascular glaucoma, comprising the step of administering to  
5 a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. In one embodiment, the compound may be administered topically to the eye in order to treat or prevent early forms of neovascular glaucoma. Within other embodiments, the compound may be implanted by injection into the region of the  
10 anterior chamber angle. Within other embodiments, the compound may also be placed in any location such that the compound is continuously released into the aqueous humor. Within another aspect of the present invention, methods are provided for treating or preventing proliferative diabetic retinopathy, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide,  
15 polypeptide, antagonist and/or agonist to the eyes, such that the formation of blood vessels is inhibited.

Within particularly preferred embodiments of the invention, proliferative diabetic retinopathy may be treated by injection into the aqueous humor or the vitreous, in order to increase the local concentration of the polynucleotide,  
20 polypeptide, antagonist and/or agonist in the retina. Preferably, this treatment should be initiated prior to the acquisition of severe disease requiring photocoagulation.

Within another aspect of the present invention, methods are provided for treating or preventing retrolental fibroplasia, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist  
25 and/or agonist to the eye, such that the formation of blood vessels is inhibited. The compound may be administered topically, via intravitreal injection and/or via intraocular implants.

Additionally, diseases, disorders, and/or conditions which can be treated, prevented, and/or diagnosed with the polynucleotides, polypeptides, agonists and/or  
30 agonists include, but are not limited to, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome,



pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

Moreover, diseases, disorders, and/or conditions and/or states, which can be treated, prevented, and/or diagnosed with the the polynucleotides, polypeptides, agonists and/or agonists include, but are not limited to, solid tumors, blood born tumors such as leukemias, tumor metastasis, Kaposi's sarcoma, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, rheumatoid arthritis, psoriasis, ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, and uveitis, delayed wound healing, endometriosis, vasculogenesis, granulations, hypertrophic scars (keloids), nonunion fractures, scleroderma, trachoma, vascular adhesions, myocardial angiogenesis, coronary collaterals, cerebral collaterals, arteriovenous malformations, ischemic limb angiogenesis, Osler-Webber Syndrome, plaque neovascularization, telangiectasia, hemophilic joints, angiofibroma, fibromuscular dysplasia, wound granulation, Crohn's disease, atherosclerosis, birth control agent by preventing vascularization required for embryo implantation controlling menstruation, diseases that have angiogenesis as a pathologic consequence such as cat scratch disease (Rochelie minalia quintosa), ulcers (*Helicobacter pylori*), Bartonellosis and bacillary angiomatosis.

In one aspect of the birth control method, an amount of the compound sufficient to block embryo implantation is administered before or after intercourse and fertilization have occurred, thus providing an effective method of birth control, possibly a "morning after" method. Polynucleotides, polypeptides, agonists and/or agonists may also be used in controlling menstruation or administered as either a peritoneal lavage fluid or for peritoneal implantation in the treatment of endometriosis.

Polynucleotides, polypeptides, agonists and/or agonists of the present invention may be incorporated into surgical sutures in order to prevent stitch granulomas.

Polynucleotides, polypeptides, agonists and/or agonists may be utilized in a wide variety of surgical procedures. For example, within one aspect of the present invention a compositions (in the form of, for example, a spray or film) may be utilized



to coat or spray an area prior to removal of a tumor, in order to isolate normal surrounding tissues from malignant tissue, and/or to prevent the spread of disease to surrounding tissues. Within other aspects of the present invention, compositions (e.g., in the form of a spray) may be delivered via endoscopic procedures in order to coat  
5 tumors, or inhibit angiogenesis in a desired locale. Within yet other aspects of the present invention, surgical meshes which have been coated with anti-angiogenic compositions of the present invention may be utilized in any procedure wherein a surgical mesh might be utilized. For example, within one embodiment of the invention a surgical mesh laden with an anti-angiogenic composition may be utilized  
10 during abdominal cancer resection surgery (e.g., subsequent to colon resection) in order to provide support to the structure, and to release an amount of the anti-angiogenic factor.

Within further aspects of the present invention, methods are provided for treating tumor excision sites, comprising administering a polynucleotide, polypeptide,  
15 agonist and/or agonist to the resection margins of a tumor subsequent to excision, such that the local recurrence of cancer and the formation of new blood vessels at the site is inhibited. Within one embodiment of the invention, the anti-angiogenic compound is administered directly to the tumor excision site (e.g., applied by swabbing, brushing or otherwise coating the resection margins of the tumor with the  
20 anti-angiogenic compound). Alternatively, the anti-angiogenic compounds may be incorporated into known surgical pastes prior to administration. Within particularly preferred embodiments of the invention, the anti-angiogenic compounds are applied after hepatic resections for malignancy, and after neurosurgical operations.

Within one aspect of the present invention, polynucleotides, polypeptides,  
25 agonists and/or agonists may be administered to the resection margin of a wide variety of tumors, including for example, breast, colon, brain and hepatic tumors. For example, within one embodiment of the invention, anti-angiogenic compounds may be administered to the site of a neurological tumor subsequent to excision, such that the formation of new blood vessels at the site are inhibited.

30 The polynucleotides, polypeptides, agonists and/or agonists of the present invention may also be administered along with other anti-angiogenic factors. Representative examples of other anti-angiogenic factors include: Anti-Invasive



Factor, retinoic acid and derivatives thereof, paclitaxel, Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

5       Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include platelet factor 4; 30 protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence



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## Diseases associated with

Diseases associated with increased cell survival or the inhibition of apoptosis that could be treated, prevented, and/or diagnosed by the polynucleotides or polypeptides and/or antagonists or agonists of the invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune diseases, disorders, and/or conditions (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. In preferred embodiments, the polynucleotides or polypeptides, and/or



agonists or antagonists of the invention are used to inhibit growth, progression, and/or metasis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be treated, prevented or diagnosed by the polynucleotides or polypeptides, or agonists or antagonists of the invention, include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be treated, prevented, and/or diagnosed by the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, include AIDS; neurodegenerative diseases, disorders, and/or conditions (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune diseases, disorders, and/or conditions (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis,



Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

### **Wound Healing and Epithelial Cell Proliferation**

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, for therapeutic purposes, for example, to stimulate epithelial cell proliferation and basal keratinocytes for the purpose of wound healing, and to stimulate hair follicle production and healing of dermal wounds. Polynucleotides or polypeptides, as well as agonists or antagonists of the invention, may be clinically useful in stimulating wound healing including surgical wounds, excisional wounds, deep wounds involving damage of the dermis and epidermis, eye tissue wounds, dental tissue wounds, oral cavity wounds, diabetic ulcers, dermal ulcers, cubitus ulcers, arterial ulcers, venous stasis ulcers, burns resulting from heat exposure or chemicals, and other abnormal wound healing conditions such as uremia, malnutrition, vitamin deficiencies and complications associated with systemic treatment with steroids, radiation therapy and antineoplastic drugs and antimetabolites. Polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to promote dermal reestablishment subsequent to dermal loss

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to increase the adherence of skin grafts to a wound bed and to stimulate re-epithelialization from the wound bed. The following are a non-exhaustive list of grafts that polynucleotides or polypeptides, agonists or antagonists of the invention, could be used to increase adherence to a wound bed: autografts, artificial skin, allografts, autodermic graft, autoepdermic grafts, avacular grafts, Blair-Brown grafts, bone graft, brephoplastic grafts, cutis graft, delayed graft, dermic graft,



epidermic graft, fascia graft, full thickness graft, heterologous graft, xenograft, homologous graft, hyperplastic graft, lamellar graft, mesh graft, mucosal graft, Ollier-Thiersch graft, omentum graft, patch graft, pedicle graft, penetrating graft, split skin graft, thick split graft. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, can be used to promote skin strength and to improve the appearance of aged skin.

It is believed that the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, will also produce changes in hepatocyte proliferation, and epithelial cell proliferation in the lung, breast, pancreas, stomach, small intestine, and large intestine. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could promote proliferation of epithelial cells such as sebocytes, hair follicles, hepatocytes, type II pneumocytes, mucin-producing goblet cells, and other epithelial cells and their progenitors contained within the skin, lung, liver, and gastrointestinal tract. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may promote proliferation of endothelial cells, keratinocytes, and basal keratinocytes.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could also be used to reduce the side effects of gut toxicity that result from radiation, chemotherapy treatments or viral infections. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may have a cytoprotective effect on the small intestine mucosa. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may also stimulate healing of mucositis (mouth ulcers) that result from chemotherapy and viral infections.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could further be used in full regeneration of skin in full and partial thickness skin defects, including burns, (i.e., repopulation of hair follicles, sweat glands, and sebaceous glands), treatment of other skin defects such as psoriasis. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to treat epidermolysis bullosa, a defect in adherence of the epidermis to the underlying dermis which results in frequent, open and painful blisters by accelerating reepithelialization of these lesions. The polynucleotides or polypeptides, and/or



agonists or antagonists of the invention, could also be used to treat gastric and duodenal ulcers and help heal by scar formation of the mucosal lining and regeneration of glandular mucosa and duodenal mucosal lining more rapidly. Inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, are diseases which result in destruction of the mucosal surface of the small or large intestine, respectively. Thus, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to promote the resurfacing of the mucosal surface to aid more rapid healing and to prevent progression of inflammatory bowel disease. Treatment with the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, is expected to have a significant effect on the production of mucus throughout the gastrointestinal tract and could be used to protect the intestinal mucosa from injurious substances that are ingested or following surgery. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to treat diseases associated with the under expression of the polynucleotides of the invention.

Moreover, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to prevent and heal damage to the lungs due to various pathological states. A growth factor such as the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, which could stimulate proliferation and differentiation and promote the repair of alveoli and bronchiolar epithelium to prevent or treat acute or chronic lung damage. For example, emphysema, which results in the progressive loss of alveoli, and inhalation injuries, i.e., resulting from smoke inhalation and burns, that cause necrosis of the bronchiolar epithelium and alveoli could be effectively treated, prevented, and/or diagnosed using the polynucleotides or polypeptides, and/or agonists or antagonists of the invention. Also, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to stimulate the proliferation of and differentiation of type II pneumocytes, which may help treat or prevent disease such as hyaline membrane diseases, such as infant respiratory distress syndrome and bronchopulmonary dysplasia, in premature infants.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could stimulate the proliferation and differentiation of hepatocytes and,



thus, could be used to alleviate or treat liver diseases and pathologies such as fulminant liver failure caused by cirrhosis, liver damage caused by viral hepatitis and toxic substances (i.e., acetaminophen, carbon tetrachloride and other hepatotoxins known in the art).

5           In addition, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used treat or prevent the onset of diabetes mellitus. In patients with newly diagnosed Types I and II diabetes, where some islet cell function remains, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to maintain the islet function so as to alleviate, delay or  
10   prevent permanent manifestation of the disease. Also, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used as an auxiliary in islet cell transplantation to improve or promote islet cell function.

#### 15   Neurological Diseases

Nervous system diseases, disorders, and/or conditions, which can be treated, prevented, and/or diagnosed with the compositions of the invention (e.g., polypeptides, polynucleotides, and/or agonists or antagonists), include, but are not limited to, nervous system injuries, and diseases, disorders, and/or conditions which  
20   result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated, prevented, and/or diagnosed in a patient (including human and non-human mammalian patients) according to the invention, include but are not limited to, the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems: (1)  
25   ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia; (2) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries; (3) malignant lesions, in which a portion of  
30   the nervous system is destroyed or injured by malignant tissue which is either a nervous system associated malignancy or a malignancy derived from non-nervous



system tissue; (4) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, syphilis; (5) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis (ALS); (6) lesions associated with nutritional diseases, disorders, and/or conditions, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar degeneration; (7) neurological lesions associated with systemic diseases including, but not limited to, diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis; (8) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and (9) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including, but not limited to, multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

In a preferred embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to protect neural cells from the damaging effects of cerebral hypoxia. According to this embodiment, the compositions of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with cerebral hypoxia. In one aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with cerebral ischemia. In another aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with cerebral infarction. In another aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose or prevent neural cell injury associated with a stroke.



In a further aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with a heart attack.

The compositions of the invention which are useful for treating or preventing a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons. For example, and not by way of limitation, compositions of the invention which elicit any of the following effects may be useful according to the invention: (1) increased survival time of neurons in culture; (2) increased sprouting of neurons in culture or *in vivo*; (3) increased production of a neuron-associated molecule in culture or *in vivo*, e.g., choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or (4) decreased symptoms of neuron dysfunction *in vivo*. Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may routinely be measured using a method set forth herein or otherwise known in the art, such as, for example, the method set forth in Arakawa et al. (J. Neurosci. 10:3507-3515 (1990)); increased sprouting of neurons may be detected by methods known in the art, such as, for example, the methods set forth in Pestronk et al. (Exp. Neurol. 70:65-82 (1980)) or Brown et al. (Ann. Rev. Neurosci. 4:17-42 (1981)); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, etc., using techniques known in the art and depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, e.g., weakness, motor neuron conduction velocity, or functional disability.

In specific embodiments, motor neuron diseases, disorders, and/or conditions that may be treated, prevented, and/or diagnosed according to the invention include, but are not limited to, diseases, disorders, and/or conditions such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as diseases, disorders, and/or conditions that selectively affect neurons such as amyotrophic lateral sclerosis, and including, but not limited to, progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile



and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

## 5 Infectious Disease

A polypeptide or polynucleotide and/or agonist or antagonist of the present invention can be used to treat, prevent, and/or diagnose infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated, prevented, and/or diagnosed. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, polypeptide or polynucleotide and/or agonist or antagonist of the present invention may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention. Examples of viruses, include, but are not limited to Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papilloma virus, Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiolitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox,



hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat patients nonresponsive to one or more other commercially available hepatitis vaccines. In a further specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose AIDS.

Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, include, but not limited to, the following Gram-Negative and Gram-positive bacteria and bacterial families and fungi: Actinomycetales (e.g., *Corynebacterium*, *Mycobacterium*, *Nocardia*), *Cryptococcus neoformans*, Aspergillosis, Bacillaceae (e.g., Anthrax, *Clostridium*), Bacteroidaceae, Blastomycosis, *Bordetella*, *Borrelia* (e.g., *Borrelia burgdorferi*), Brucellosis, Candidiasis, *Campylobacter*, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, *E. coli* (e.g., Enterotoxigenic *E. coli* and Enterohemorrhagic *E. coli*), Enterobacteriaceae (*Klebsiella*, *Salmonella* (e.g., *Salmonella typhi*, and *Salmonella paratyphi*), *Serratia*, *Yersinia*), Erysipelothrix, *Helicobacter*, Legionellosis, Leptospirosis, *Listeria*, Mycoplasmatales, *Mycobacterium leprae*, *Vibrio cholerae*, Neisseriaceae (e.g., *Acinetobacter*, Gonorrhea, Meningococcal), *Meisseria meningitidis*, Pasteurellaceae Infections (e.g., *Actinobacillus*, *Heamophilus* (e.g., *Heamophilus influenza type B*), *Pasteurella*), *Pseudomonas*, Rickettsiaceae, Chlamydiaceae, Syphilis, *Shigella spp.*, Staphylococcal, Meningiococcal, Pneumococcal and Streptococcal (e.g., *Streptococcus pneumoniae* and Group B Streptococcus). These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis),



gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis (e.g., meningitis types A and B), Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections. Polynucleotides or polypeptides, agonists or antagonists of the invention, can be used to treat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, agonists or antagonists of the invention are used to treat, prevent, and/or diagnose: tetanus, Diphtheria, botulism, and/or meningitis type B.

Moreover, parasitic agents causing disease or symptoms that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans (e.g., Plasmodium virax, Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose malaria.

Preferably, treatment or prevention using a polypeptide or polynucleotide and/or agonist or antagonist of the present invention could either be by administering an effective amount of a polypeptide to the patient, or by removing cells from the patient, supplying the cells with a polynucleotide of the present invention, and



returning the engineered cells to the patient (ex vivo therapy). Moreover, the polypeptide or polynucleotide of the present invention can be used as an antigen in a vaccine to raise an immune response against infectious disease.

## 5 **Regeneration**

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues. (See, Science 276:59-87 (1997).) The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, 10 trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteoarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal 15 or cardiac), vasculature (including vascular and lymphatics), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase regeneration of tissues difficult to heal. For example, 20 increased tendon/ligament regeneration would quicken recovery time after damage.

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated, prevented, and/or diagnosed include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue 25 regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using a polynucleotide or polypeptide and/or agonist or antagonist of the present invention to proliferate and differentiate nerve cells. Diseases that could be treated, prevented, 30 and/or diagnosed using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic diseases, disorders, and/or conditions (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and



- stroke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated, prevented, and/or diagnosed using the polynucleotide or polypeptide and/or agonist or antagonist of the present invention.

### **Chemotaxis**

- A polynucleotide or polypeptide and/or agonist or antagonist of the present invention may have chemotaxis activity. A chemotactic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

- A polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase chemotactic activity of particular cells. These chemotactic molecules can then be used to treat, prevent, and/or diagnose inflammation, infection, hyperproliferative diseases, disorders, and/or conditions, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotactic molecules can be used to treat, prevent, and/or diagnose wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat, prevent, and/or diagnose wounds.

- It is also contemplated that a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may inhibit chemotactic activity. These molecules could also be used to treat, prevent, and/or diagnose diseases, disorders, and/or conditions. Thus, a polynucleotide or polypeptide and/or agonist or antagonist of the present invention could be used as an inhibitor of chemotaxis.

### **Binding Activity**

- A polypeptide of the present invention may be used to screen for molecules that bind to the polypeptide or for molecules to which the polypeptide binds. The



binding of the polypeptide and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the polypeptide or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

- 5            Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991).) Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds, or at least, a fragment of the receptor capable of being bound by the polypeptide (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

- Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, *Drosophila*, or *E. coli*.  
15       Cells expressing the polypeptide (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either the polypeptide or the molecule.

- The assay may simply test binding of a candidate compound to the  
20       polypeptide, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

- Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product  
25       mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

- Preferably, an ELISA assay can measure polypeptide level or activity in a  
30       sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure polypeptide level or activity by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.



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24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of polynucleotides and corresponding polypeptides of the invention may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired polynucleotide sequence of the invention molecule by homologous, or site-specific, recombination. In another embodiment, polynucleotides and corresponding polypeptides of the invention may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of the polypeptides of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules. In preferred embodiments, the heterologous molecules are family members. In further preferred embodiments, the heterologous molecule is a growth factor such as, for example, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I), transforming growth factor (TGF)-alpha, epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF-beta, bone morphogenetic protein (BMP)-2, BMP-4, BMP-5, BMP-6, BMP-7, activins A and B, decapentaplegic(dpp), 60A, OP-2, dorsalin, growth differentiation factors (GDFs), nodal, MIS, inhibin-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta5, and glial-derived neurotrophic factor (GDNF).

Other preferred fragments are biologically active fragments of the polypeptides of the invention. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Additionally, this invention provides a method of screening compounds to identify those which modulate the action of the polypeptide of the present invention. An example of such an assay comprises combining a mammalian fibroblast cell, a the polypeptide of the present invention, the compound to be screened and  $^3\text{H}$  thymidine under cell culture conditions where the fibroblast cell would normally proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of fibroblast proliferation in the presence of the



compound to determine if the compound stimulates proliferation by determining the uptake of 3[H] thymidine in each case. The amount of fibroblast cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of 3[H] thymidine. Both agonist and antagonist compounds may be identified by this procedure.

In another method, a mammalian cell or membrane preparation expressing a receptor for a polypeptide of the present invention is incubated with a labeled polypeptide of the present invention in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following interaction of a compound to be screened and the receptor is measured and the ability of the compound to bind to the receptor and elicit a second messenger response is measured to determine if the compound is a potential agonist or antagonist. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat, prevent, and/or diagnose disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the polypeptides of the invention from suitably manipulated cells or tissues. Therefore, the invention includes a method of identifying compounds which bind to the polypeptides of the invention comprising the steps of: (a) incubating a candidate binding compound with the polypeptide; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with the polypeptide, (b) assaying a biological activity, and (b) determining if a biological activity of the polypeptide has been altered.

Also, one could identify molecules bind a polypeptide of the invention experimentally by using the beta-pleated sheet regions contained in the polypeptide sequence of the protein. Accordingly, specific embodiments of the invention are directed to polynucleotides encoding polypeptides which comprise, or alternatively



consist of, the amino acid sequence of each beta pleated sheet regions in a disclosed polypeptide sequence. Additional embodiments of the invention are directed to polynucleotides encoding polypeptides which comprise, or alternatively consist of, any combination or all of contained in the polypeptide sequences of the invention.

- 5 Additional preferred embodiments of the invention are directed to polypeptides which comprise, or alternatively consist of, the amino acid sequence of each of the beta pleated sheet regions in one of the polypeptide sequences of the invention. Additional embodiments of the invention are directed to polypeptides which comprise, or alternatively consist of, any combination or all of the beta pleated sheet regions in one
- 10 of the polypeptide sequences of the invention.

#### **Targeted Delivery**

- In another embodiment, the invention provides a method of delivering compositions to targeted cells expressing a receptor for a polypeptide of the invention,
- 15 or cells expressing a cell bound form of a polypeptide of the invention.

- As discussed herein, polypeptides or antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions. In one embodiment, the invention provides a method for the specific delivery of
- 20 compositions of the invention to cells by administering polypeptides of the invention (including antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double
- 25 stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

- In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention (e.g., polypeptides of the invention or antibodies of the invention) in
- 30 association with toxins or cytotoxic prodrugs.

By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of



toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing  
5 containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. By "cytotoxic prodrug" is meant a non-toxic compound that is converted by an enzyme, normally present in the cell, into  
10 a cytotoxic compound. Cytotoxic prodrugs that may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxyacetamide derivatives of doxorubicin.

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### **Drug Screening**

Further contemplated is the use of the polypeptides of the present invention, or the polynucleotides encoding these polypeptides, to screen for molecules which modify the activities of the polypeptides of the present invention. Such a method  
20 would include contacting the polypeptide of the present invention with a selected compound(s) suspected of having antagonist or agonist activity, and assaying the activity of these polypeptides following binding.

This invention is particularly useful for screening therapeutic compounds by using the polypeptides of the present invention, or binding fragments thereof, in any  
25 of a variety of drug screening techniques. The polypeptide or fragment employed in such a test may be affixed to a solid support, expressed on a cell surface, free in solution, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or fragment. Drugs are screened against such transformed  
30 cells in competitive binding assays. One may measure, for example, the formulation of complexes between the agent being tested and a polypeptide of the present invention.



Thus, the present invention provides methods of screening for drugs or any other agents which affect activities mediated by the polypeptides of the present invention. These methods comprise contacting such an agent with a polypeptide of the present invention or a fragment thereof and assaying for the presence of a complex  
5 between the agent and the polypeptide or a fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen are typically labeled. Following incubation, free agent is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of a particular agent to bind to the polypeptides of the present invention.

10 Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the polypeptides of the present invention, and is described in great detail in European Patent Application 84/03564, published on September 13, 1984, which is incorporated herein by reference herein. Briefly stated, large numbers of different small peptide test compounds are  
15 synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptides of the present invention and washed. Bound polypeptides are then detected by methods well known in the art. Purified polypeptides are coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies may be used to  
20 capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding polypeptides of the present invention specifically compete with a test compound for binding to the polypeptides or fragments thereof. In this manner, the antibodies are used to detect the presence of  
25 any peptide which shares one or more antigenic epitopes with a polypeptide of the invention.

#### **Polypeptides of the Invention Binding Peptides and Other Molecules**

The invention also encompasses screening methods for identifying  
30 polypeptides and nonpolypeptides that bind polypeptides of the invention, and the polypeptide of the invention binding molecules identified thereby. These binding molecules are useful, for example, as agonists and antagonists of the polypeptides of



the invention. Such agonists and antagonists can be used, in accordance with the invention, in the therapeutic embodiments described in detail, below.

This method comprises the steps of:

- a. contacting a polypeptide of the invention with a plurality of molecules; and
- 5 b. identifying a molecule that binds the polypeptide of the invention.

The step of contacting the polypeptide of the invention with the plurality of molecules may be effected in a number of ways. For example, one may contemplate immobilizing the polypeptide of the invention on a solid support and bringing a solution of the plurality of molecules in contact with the immobilized polypeptide of the invention. Such a procedure would be akin to an affinity chromatographic process, with the affinity matrix being comprised of the immobilized polypeptide of the invention. The molecules having a selective affinity for the polypeptide of the invention can then be purified by affinity selection. The nature of the solid support, process for attachment of the polypeptide of the invention to the solid support, solvent, and conditions of the affinity isolation or selection are largely conventional and well known to those of ordinary skill in the art.

Alternatively, one may also separate a plurality of polypeptides into substantially separate fractions comprising a subset of or individual polypeptides. For instance, one can separate the plurality of polypeptides by gel electrophoresis, column chromatography, or like method known to those of ordinary skill for the separation of polypeptides. The individual polypeptides can also be produced by a transformed host cell in such a way as to be expressed on or about its outer surface (e.g., a recombinant phage). Individual isolates can then be "probed" by the polypeptide of the invention, optionally in the presence of an inducer should one be required for expression, to determine if any selective affinity interaction takes place between the polypeptide of the invention and the individual clone. Prior to contacting the polypeptide of the invention with each fraction comprising individual polypeptides, the polypeptides could first be transferred to a solid support for additional convenience. Such a solid support may simply be a piece of filter membrane, such as one made of nitrocellulose or nylon. In this manner, positive clones could be identified from a collection of transformed host cells of an expression library, which harbor a DNA construct encoding a polypeptide having a selective affinity for a polypeptide of the invention.







In vitro translation-based libraries include but are not limited to those described in PCT Publication No. WO 91/05058 dated Apr. 18, 1991; and Mattheakis et al., 1994, Proc. Natl. Acad. Sci. USA 91:9022-9026.

By way of examples of nonpeptide libraries, a benzodiazepine library (see  
5 e.g., Bunin et al., 1994, Proc. Natl. Acad. Sci. USA 91:4708-4712) can be adapted for use. Peptoid libraries (Simon et al., 1992, Proc. Natl. Acad. Sci. USA 89:9367-9371) can also be used. Another example of a library that can be used, in which the amide functionalities in peptides have been permethylated to generate a chemically transformed combinatorial library, is described by Ostresh et al. (1994, Proc. Natl.  
10 Acad. Sci. USA 91:11138-11142).

The variety of non-peptide libraries that are useful in the present invention is great. For example, Ecker and Crooke, 1995, Bio/Technology 13:351-360 list benzodiazepines, hydantoins, piperazinediones, biphenyls, sugar analogs, beta-mercaptoketones, arylacetic acids, acylpiperidines, benzopyrans, cubanes, xanthines,  
15 aminimides, and oxazolones as among the chemical species that form the basis of various libraries.

Non-peptide libraries can be classified broadly into two types: decorated monomers and oligomers. Decorated monomer libraries employ a relatively simple scaffold structure upon which a variety functional groups is added. Often the scaffold  
20 will be a molecule with a known useful pharmacological activity. For example, the scaffold might be the benzodiazepine structure.

Non-peptide oligomer libraries utilize a large number of monomers that are assembled together in ways that create new shapes that depend on the order of the monomers. Among the monomer units that have been used are carbamates,  
25 pyrrolinones, and morpholinos. Peptoids, peptide-like oligomers in which the side chain is attached to the alpha amino group rather than the alpha carbon, form the basis of another version of non-peptide oligomer libraries. The first non-peptide oligomer libraries utilized a single type of monomer and thus contained a repeating backbone. Recent libraries have utilized more than one monomer, giving the libraries added  
30 flexibility.

Screening the libraries can be accomplished by any of a variety of commonly known methods. See, e.g., the following references, which disclose screening of



peptide libraries: Parmley and Smith, 1989, *Adv. Exp. Med. Biol.* 251:215-218; Scott and Smith, 1990, *Science* 249:386-390; Fowlkes et al., 1992, *BioTechniques* 13:422-427; Oldenburg et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:5393-5397; Yu et al., 1994, *Cell* 76:933-945; Staudt et al., 1988, *Science* 241:577-580; Bock et al., 1992, 5 *Nature* 355:564-566; Tuerk et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:6988-6992; Ellington et al., 1992, *Nature* 355:850-852; U.S. Pat. No. 5,096,815, U.S. Pat. No. 5,223,409, and U.S. Pat. No. 5,198,346, all to Ladner et al.; Rebar and Pabo, 1993, *Science* 263:671-673; and CT Publication No. WO 94/18318.

In a specific embodiment, screening to identify a molecule that binds a 10 polypeptide of the invention can be carried out by contacting the library members with a polypeptide of the invention immobilized on a solid phase and harvesting those library members that bind to the polypeptide of the invention. Examples of such screening methods, termed "panning" techniques are described by way of example in Parmley and Smith, 1988, *Gene* 73:305-318; Fowlkes et al., 1992, *BioTechniques* 15 13:422-427; PCT Publication No. WO 94/18318; and in references cited herein.

In another embodiment, the two-hybrid system for selecting interacting proteins in yeast (Fields and Song, 1989, *Nature* 340:245-246; Chien et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:9578-9582) can be used to identify molecules that specifically bind to a polypeptide of the invention.

20 Where the polypeptide of the invention binding molecule is a polypeptide, the polypeptide can be conveniently selected from any peptide library, including random peptide libraries, combinatorial peptide libraries, or biased peptide libraries. The term "biased" is used herein to mean that the method of generating the library is manipulated so as to restrict one or more parameters that govern the diversity of the 25 resulting collection of molecules, in this case peptides.

Thus, a truly random peptide library would generate a collection of peptides in which the probability of finding a particular amino acid at a given position of the peptide is the same for all 20 amino acids. A bias can be introduced into the library, however, by specifying, for example, that a lysine occur every fifth amino acid or that 30 positions 4, 8, and 9 of a decapeptide library be fixed to include only arginine. Clearly, many types of biases can be contemplated, and the present invention is not restricted to any particular bias. Furthermore, the present invention contemplates



As mentioned above, in the case of a polypeptide of the invention binding molecule that is a polypeptide, the polypeptide may have about 6 to less than about 60 amino acid residues, preferably about 6 to about 10 amino acid residues, and most preferably, about 6 to about 22 amino acids. In another embodiment, a polypeptide of the invention binding polypeptide has in the range of 15-100 amino acids, or 20-50 amino acids.

The selected polypeptide of the invention binding polypeptide can be obtained  
10 by chemical synthesis or recombinant expression.

### Antisense And Ribozyme (Antagonists)

In specific embodiments, antagonists according to the present invention are nucleic acids corresponding to the sequences contained in SEQ ID NO:X, or the complementary strand thereof, and/or to nucleotide sequences contained a deposited clone. In one embodiment, antisense sequence is generated internally by the organism, in another embodiment, the antisense sequence is separately administered (see, for example, O'Connor, *Neurochem.*, 56:560 (1991). *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988).

Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, *Neurochem.*, 56:560 (1991); *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance, Lee et al., *Nucleic Acids Research*, 6:3073 (1979); Cooney et al., *Science*, 241:456 (1988); and Dervan et al., *Science*, 251:1300 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the use of c-myc and c-myb antisense RNA constructs to inhibit the growth of the non-lymphocytic leukemia cell line HL-60 and other cell lines was previously described. (Wickstrom et al. (1988); Anfossi et al. (1989)). These experiments were performed in vitro by incubating cells with the oligoribonucleotide. A similar procedure for in vivo use is described in WO 91/15580. Briefly, a pair of



oligonucleotides for a given antisense RNA is produced as follows: A sequence complimentary to the first 15 bases of the open reading frame is flanked by an EcoRI site on the 5' end and a HindIII site on the 3' end. Next, the pair of oligonucleotides is heated at 90°C for one minute and then annealed in 2X ligation buffer (20mM TRIS HCl pH 7.5, 10mM MgCl<sub>2</sub>, 10mM dithiothreitol (DTT) and 0.2 mM ATP) and then ligated to the EcoRI/Hind III site of the retroviral vector PMV7 (WO 91/15580).

For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into receptor polypeptide.

In one embodiment, the antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the antisense nucleic acid of the invention. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding a polypeptide of the invention, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, *Nature*, 29:304-310 (1981), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell*, 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., *Proc. Natl. Acad. Sci. U.S.A.*, 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster et al., *Nature*, 296:39-42 (1982)), etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a gene of interest.



However, absolute complementarity, although preferred, is not required. A sequence “complementary to at least a portion of an RNA,” referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded antisense nucleic acids of the invention, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with a RNA sequence of the invention it may contain and still form a stable duplex (or triplex as the case may be).

One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5' end of the message, *e.g.*, the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., *Nature*, 372:333-335 (1994). Thus, oligonucleotides complementary to either the 5' - or 3' - non- translated, non-coding regions of a polynucleotide sequence of the invention could be used in an antisense approach to inhibit translation of endogenous mRNA.

Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5' -, 3' - or coding region of mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (*e.g.*,



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phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an a-anomeric oligonucleotide. An a-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gautier et al., Nucl. Acids Res., 15:6625-6641 (1987)). The oligonucleotide is a 2-O-methylribonucleotide (Inoue et al., Nucl. Acids Res., 15:6131-6148 (1987)), or a chimeric RNA-DNA analogue (Inoue et al., FEBS Lett. 215:327-330 (1987)).

Polynucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (Nucl. Acids Res., 16:3209 (1988)), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., Proc. Natl. Acad. Sci. U.S.A., 85:7448-7451 (1988)), etc.

While antisense nucleotides complementary to the coding region sequence of the invention could be used, those complementary to the transcribed untranslated region are most preferred.

Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, Science, 247:1222-1225 (1990)). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs corresponding to the polynucleotides of the invention, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5' -UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature, 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within each nucleotide sequence disclosed in the sequence listing. Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5'



As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells which express the polynucleotides of the invention in vivo. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

20           The antagonist/agonist may also be employed to prevent hyper-vascular diseases, and prevent the proliferation of epithelial lens cells after extracapsular cataract surgery. Prevention of the mitogenic activity of the polypeptides of the present invention may also be desirable in cases such as restenosis after balloon angioplasty.

The antagonist/agonist may also be employed to treat, prevent, and/or diagnose the diseases described herein.

Thus, the invention provides a method of treating or preventing diseases, disorders, and/or conditions, including but not limited to the diseases, disorders, and/or conditions listed throughout this application, associated with overexpression of a polynucleotide of the present invention by administering to a patient (a) an antisense



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The polypeptide of the invention may also be employed to maintain organs before transplantation or for supporting cell culture of primary tissues.



The polypeptide of the present invention may also be employed for inducing tissue of mesodermal origin to differentiate in early embryos.

5 The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

10 The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, polypeptides or polynucleotides and/or agonist or antagonists of the present invention may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

15 Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may be used to change a mammal's mental state or physical state by influencing biorhythms, cardiac rhythms, depression (including depressive diseases, disorders, and/or conditions), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

20 Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

## 25 **Other Preferred Embodiments**

Other preferred embodiments of the claimed invention include an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 50 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1.

30 Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of



positions beginning with the nucleotide at about the position of the 5' Nucleotide of the Clone Sequence and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous  
5 nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of  
positions beginning with the nucleotide at about the position of the 5' Nucleotide of  
the Start Codon and ending with the nucleotide at about the position of the 3'  
Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Similarly preferred is a nucleic acid molecule wherein said sequence of  
10 contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the  
range of positions beginning with the nucleotide at about the position of the 5'  
Nucleotide of the First Amino Acid of the Signal Peptide and ending with the  
nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as  
defined for SEQ ID NO:X in Table 1.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 150 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

Further preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 500 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

A further preferred embodiment is a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the nucleotide sequence of SEQ ID NO:X beginning with the nucleotide at about the position of the 5' Nucleotide of the First Amino Acid of the Signal Peptide and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence of SEQ ID NO:X.

Also preferred is an isolated nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule, wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization



conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

Also preferred is a composition of matter comprising a DNA molecule which comprises a human cDNA clone identified by a cDNA Clone Identifier in Table 1,  
5 which DNA molecule is contained in the material deposited with the American Type Culture Collection and given the ATCC Deposit Number shown in Table 1 for said cDNA Clone Identifier.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous  
10 nucleotides in the nucleotide sequence of a human cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in the deposit given the ATCC Deposit Number shown in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said sequence of at least 50 contiguous nucleotides is included in the nucleotide sequence of the  
15 complete open reading frame sequence encoded by said human cDNA clone.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 150 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule  
20 comprising a nucleotide sequence which is at least 95% identical to sequence of at least 500 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete  
25 nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is a method for detecting in a biological sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X  
30 wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table



Also preferred is the above method wherein said step of comparing sequences comprises determining the extent of nucleic acid hybridization between nucleic acid molecules in said sample and a nucleic acid molecule comprising said sequence selected from said group. Similarly, also preferred is the above method wherein said step of comparing sequences is performed by comparing the nucleotide sequence determined from a nucleic acid molecule in said sample with said sequence selected from said group. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

The method for identifying the species, tissue or cell type of a biological sample can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject nucleic acid molecules, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a



nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1;  
and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA  
Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit  
Number shown for said cDNA clone in Table 1.

5           The method for diagnosing a pathological condition can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1.

Also preferred is a polypeptide, wherein said sequence of contiguous amino acids is included in the amino acid sequence of SEQ ID NO:Y in the range of positions beginning with the residue at about the position of the First Amino Acid of the Secreted Portion and ending with the residue at about the Last Amino Acid of the Open Reading Frame as set forth for SEQ ID NO:Y in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.



Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid  
5 sequence at least 95% identical to the complete amino acid sequence of SEQ ID  
NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is a polypeptide wherein said sequence of contiguous amino acids is included in the amino acid sequence of a secreted portion of the secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.



Further preferred is an isolated antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is a method for detecting in a biological sample a polypeptide comprising an amino acid sequence which is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group and determining whether the sequence of said polypeptide molecule in said sample is at least 90% identical to said sequence of at least 10 contiguous amino acids.

Also preferred is the above method wherein said step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group comprises determining the extent of specific binding of polypeptides in said sample to an antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is the above method wherein said step of comparing sequences is performed by comparing the amino acid sequence determined from a polypeptide molecule in said sample with said sequence selected from said group.



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polypeptide in a prokaryotic host.

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clone in Table 1. The isolated polypeptide produced by this method is also preferred.



Also preferred is a method of treatment of an individual in need of an increased level of a secreted protein activity, which method comprises administering to such an individual a pharmaceutical composition comprising an amount of an isolated polypeptide, polynucleotide, or antibody of the claimed invention effective to  
 5 increase the level of said protein activity in said individual.

The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-human primate, and human. In specific embodiments, the host is a mouse, rabbit,  
 10 goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

In specific embodiments of the invention, for each "Contig ID" listed in the fourth column of Table 6, preferably excluded are one or more polynucleotides  
 15 comprising, or alternatively consisting of, a nucleotide sequence referenced in the fifth column of Table 6 and described by the general formula of a-b, whereas a and b are uniquely determined for the corresponding SEQ ID NO:X referred to in column 3 of Table 6. Further specific embodiments are directed to polynucleotide sequences excluding one, two, three, four, or more of the specific polynucleotide sequences  
 20 referred to in the fifth column of Table 6. In no way is this listing meant to encompass all of the sequences which may be excluded by the general formula, it is just a representative example. All references available through these accessions are hereby incorporated by reference in their entirety.

TABLE 6

Gene No.	cDNA Clone ID	NT SEQ ID NO: X	Contig ID	Public Accession Numbers
1	HTDAA93	11	839446	AI817099, AA481246, AI568200, AA973640, AA554541, AI291110, AI017230, AI623385, H08023, AA761091, T34379, AI221749, AA419534, AI221756, T19441, AI682007, R81743, AW298440, AI141990, AA687813, T19440, AA193482, Z39973, R81500, Z43916,



				<p> T34380, AI350408, AI698802, AA971074,  AA588098, AA481169, AW118237, D45630,  AL119399, AL119457, AL042544, AL042382,  AL119511, AL119324, AL043152, AL079794,  AI458053, AL043168, AI818980, AI579901,  AL037081, AI570807, AW166583, AI815855,  AI874261, AI619502, AI352497, AI677796,  AI648509, AI802542, AI433157, AI702073,  AW026882, AI633125, AL121365, AI583065,  AI671642, AI915291, AI699865, AI866090,  AI923370, AI698391, AW152182, AW131294,  AI670009, AI673363, AI889189, AI627988,  AI819976, AW118518, AI817543, AL045500,  AI500061, AI473799, AI288305, AW129659,  AI932794, AW051258, AI582932, AW166903,  AW051088, AW151136, AI270706, AI587606,  AI611738, AW103878, AI564719, AL079963,  AI640729, AI670002, W74529, AI367680,  AW161156, AI254731, AW081036, AI432030,  AI499285, AI635067, AL079741, AI926790,  AI866751, AI247293, AI890833, AI828682,  AI473536, AA806720, AI366900, AI619426,  AI536638, AI932966, AI694157, AI818683,  AI564290, AI610895, F27788, AI637584,  AW193911, AI431327, AI559296, AW167228,  AI923124, AW104827, AW104724, AI291601,  AI473554, AW080746, AI269862, AL037582,  AL037602, AI934259, AI624543, AI571439,  AI452560, AI926367, AI521040, AI640873,  AI909697, AI364788, AI635016, AI445025,  AW198090, AI269205, AI824576, AI933589,  AI887396, AI871697, AI815232, AL120853,  AW080090, AI491775, AI625079, AI581033,  AW148363, AI355827, AW050850, AI571867,  AI567128, AI683173, N33175, AI687362,  AI758812, AI440239, AI635461, AI686817,  R32821, AW075667, AI591075, AI554821,  AW148408, AL121270, AI280637, AW090393,  AW190194, AI687728, AW090550, AI469112,  AI471909, AI887775, AI628331, AW193530,  AW073270, AW129230, AW169604, AI610690,  AW078712, AI862139, AI521560, AI682971,  AI469532, AI819326, AI866801, AI536685,  AL045774, AI580190, AI682798, AW151893,  AW235482, AI634345, AI435641, AW087207,  AW149925, AI539771, AL043975, AI254727,  AI687127, AW079572, AI912356, AI923989,  AI590043, AI609589, AI921464, AI956080,  AI636588, AL048323, AW029197, AI954183,  AI609375, AI249962, AW129722, AI537244,  AI872154, AI587114, AL036403, AI440448,  AI524677, AI569583, AI799470, AI445432,  AI591420, AW075381, AI521103, AI285448,  AI620284, AL048340, AI635467, AI445992,  AW192652, AI538259, AI569975, AI537677,  AI866770, AW132056, AI439452, X85991,  AC007227, U77594, Y11587, I89947, I48978, </p>
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				A77033, A77035, AL133080, X82434, AL080159, AF177401, A08910, A08909, AJ000937, U35846, AF113694, A08916, Z82022, AL050149, A08913, A58524, A58523, I48979, AL122049, AL137550, AL049430, AF090903, Z37987, AL133560, AF087943, AF111112, A65341, AF111849, A93350, AL137560, I33392, Y14314, AL133072, AF113677, I89931, AL137271, I49625, AL117460, AL137533, AL137478, AF183393, A08912, X65873, AR038854, AL133112, AF026816, Y16645, AL049283, E02349, AL133075, AL133568, AF090901, AL110225, X98834, S68736, AL117435, Y10655, AF113019, AL137283, AF067728, AL110221, AL110280, AL137463, AL050138, AF091084, AF097996, AF153205, AF090886, I00734, AF106862, AF078844, E06743, AL117440, A03736, AL137480, AJ012755, AL137479, AL050277, AF113689, AL137557, AL050024, A08908, AL050172, AL122098, AF026124, AF090900, Y09972, AL117457, AL133016, AF158248, U49434, AL122110, A65340, AL133640, X70685, AL049314, AL133077, AL137521, X93495, AR029490, AR013797, I03321, AL049382, AL137538, AL117394, AL133637, AF118090, AL137529, AF125948, AL050146, AF185576, AF118070, AF079763, AL050116, AF061981, M92439, S76508, A18777, AL122050, AF113699, AL049452, A76335, E00617, E00717, E00778, E07108, AL050393, X72889, S61953, E05822, U67958, AL110196, AL122111, I09499, AL096744, U80742, AL080148, AL133113, AR011880, E07361, AF100781, AL049938, AL049466, AF111851, U58996, AF028823, Y07905, AF113691, AL137476, AF003737, AL133067, AF118064, Y11254, AR000496, AF132676, U39656, AF061836, AL137705, S78214, AL133565, AL137292, AL110218, AF090934, AF017437, S36676, AL137459, X84990, AF017152, AL137256, AL050108, E15569, AF090896, AL137488, AF079765, U88966, AL080124, AF119337, AF118094, I09360, I42402, AL117583, X96540, AL117585, AL133557, L31396, U68387, AF146568, L31397, AF057300, AF057299, AL122123, AL122121, AF058921, U00763, E03348, AF031147, D16301, A12297, AJ006417, A08911, AF162270, AL137429, AR038969, AL117626, AF210052, AL133098, AL122100, AL133665, AL137658, AL122093, AL137527, AF139986, AJ005690, U72620, I89934, I89944, AF113690, S63521, Y10936, AL133081, AF104032, I46765, AR059958, AJ238278, AF061795, AF151685, AF113013, X63574, A18788, AL049464, AL080074, I26207, L19437, X76228, AL096751, and AL110222.
2	HWJAE49	12	843585	AA587273, AI470221, AI566249, AI963941,



				AI916929, AA933987, AI656124, AI914774, AA320217, AA343629, AA401047, AA400995, AL119335, AB008927, AB008390, AF055982, AB009849, AF095742, AF095743, AB012761, and AB010780.
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8	HBXFL29	18	842802	AA608680, AL135214, AI346426, AW369825, AA704114, AI953494, AA102088, AA099340, AA875957, AA411819, AW103703, AI339566, AI610736, W27706, AA825903, AI934820, AI080375, R67711, N40031, AA315231, N27293, AA401638, AI307801, Z42700, AA382141, Z38860, AI382965, AA774224, R43562, R62663, AI244553, AW118387, R62613, R22885, T35989, R66107, AI204282, F10296, AA095193, AA093900, AI964066, AW025279, AI625444, AI679506, AA587120, AB020705, AC005207, AF017152, X99717, AJ238278, E12580,



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10	HWHGP71	42	839250	AA927633, H85594, AA019612, AF190901, D89079, and U41070.
11	HLWCU38	21	828397	AI004675, AI917503, AW004064, AI472000,



				AI769374, AI796095, AI521161, AW081955, AW418833, AI393067, AI239708, AI762783, AI150276, AI167968, AW161411, AA992376, AA815053, AA808590, AW162916, AI024043, AI820073, AI492033, AI479792, AA434432, AA663539, AI188140, AI967978, AI742548, AA434205, AI523700, AI953131, W01493, R91767, AW028459, AA917611, AA954498, AW136291, AA337228, AW068312, N31370, AI198049, N35562, AI470893, T32310, W74054, AA336491, AI696080, AI825065, AA296716, AI969755, AA689578, Z43990, T34562, AA358370, T82239, AA299518, AA741139, AA931970, AA670110, AI878922, AW405776, AA314926, H61278, T08096, R69222, AA143182, R69665, AW406629, R34095, W39571, AA722481, R69651, AA308656, AA127483, C14682, D54720, C15010, AW270543, T34508, C14725, R60449, W07641, W56082, AA293093, AA962193, AL037022, AA203703, AA150033, AW163069, T31447, W05298, N42456, AI525463, T30510, AA127439, D56473, W94446, AW270463, AA460187, R13312, AA652108, AA207275, AA652058, D55229, R87091, N44268, T78786, AA187900, N48438, W56832, AA214478, AA652002, R06047, W96094, AA641037, W73910, T79919, AA092145, AA216107, N32723, T31952, N45944, N56067, AA101596, D55467, D54863, AA777569, AI313152, W00426, AA307412, R26309, W79164, N27572, T36018, W84806, AA091793, T32168, AA689439, T35524, H54579, AA195507, D54356, R09273, T32246, Z42657, AI626013, AI363793, AA652867, AA041447, AI204563, AW327264, T32471, T30738, D25641, T33952, T34750, AI193283, W67312, AI184824, W74771, AI146925, D56228, AA143061, AI041813, AI879014, D20070, N56426, AA120893, W94261, T32152, AI362989, N36393, AI557149, AA834320, AI091789, AW089921, W31087, W84770, N98523, AW157100, R15535, AA655035, W68841, AA775415, AI268669, AI086525, AA460577, N56045, AI076151, D56613, AA041392, T29957, AA295443, W74292, AF131854, L76416, X99585, U89439, AF067826, AF067824, AC006137, AL031983, AL031133, AF067825, Z98050, D16928, and X95225.
12	HMTAX46	22	839474	AA430976, AA363410, Z57532, and AP000355.
13	HIBEU15,	43	842696	H05918, H22823, AA351918, AW051876, W70283, W75978, H22824, and H13018.
14	HDPQV66	24	845979	W40332, AW002378, AW390042, AW378921, AA436105, AI784141, H23401, AA738097, AW088605, AW022494, AW020288, AW022542, AW020144, AW021084, AW020689, AI433008, AA129746, AL043289, AI801325, AA832077, AL079447, AW020592, AW083846, AL048499, AW192241, AA601333, AI064787, AW079659,



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				AI887241, AW079768, AA642295, AI696714, AI289791, AI016656, AI699020, AI491710, AI859644, AI917951, AL042753, AI915295, AI868200, AI636788, AI446720, AI690813, AW194014, AW172723, AI471429, AW087217, AI648699, AI285439, AW020455, AI305157, AI345396, AL040100, AI439664, AI559863, AW151979, AI680504, AA743474, AL047039, AW160760, AI872315, AL035847, AA737649, AI439324, AI567293, AI886355, AW248417, AL121328, AA737665, AA745650, AL138455, AI799239, AI909642, AW263569, AI952645, AC007360, AL121588, AC002527, AL035454, AC002416, AC006299, AC018767, AC006112, AC007392, AL133312, AC005157, AC005224, AL133445, U73648, AC002037, AC002564, AC004465, AC006019, AC006479, AC002538, AC004686, D83989, S75201, AL049795, AC002192, D38178, AC005564, U69730, U95739, AC006221, AL008728, AC004989, AL033523, AL096776, AC005250, AC006336, AL022315, AC006004, AC005091, AL122021, AC006978, AL022147, AC005048, AC006236, AC003977, AC004547, Z94277, AL022722, AC004066, AL034400, AC004690, AC007877, AC007193, AC006501, AC008067, AC004594, AC004057, AC006466, AC005341, AC005411, AC007151, AC006222, AL137100, AC007172, AL022165, AC007056, AC004485, AC000052, AC005296, AC005291, AC007298, AC005181, AC004019, U66059, AC009233, AC006255, AC005057, AC002289, AC007707, AC004837, J05043, AC006046, AL080245, AC000119, AC006475, AC005102, AP000025, AL035587, Z82201, AC002377, AC006213, AC016027, AC007773, AL135744, AC002385, AC007632, AB015752, AC006203, AF053645, AC004193, U51560, Z82250, AC004213, AC003032, AC002540, AC005353, AF179633, AC005886, AL022723, AL049553, U85195, AE000658, I41145, AL034374, AR068466, AC005365, AL022170, X54175, AC003029, AC005209, AC004822, X58156, AL031054, AP000458, X66401, AC005008, AL021393, AC004797, Z83840, AC007390, AC004027, AC007666, AC000004, AC005968, AC006058, Z98049, AL009029, AC005488, AC002287, AL049776, X87344, AC006371, Y10196, AC008372, AL030998, AP000517, AC004170, AC006292, AC006963, AC007880, AC002464, AL121603, AF210052, AF057280, Z80896, AC009320, Z84814, AC005519, AC005599, Z82198, AC007012, AC005701, AL133070, AB023054, AP000310, AC006120, S78214, L78779, AC004402, AC002471, AC005374, AC003677, AC004084, AL031390, AC002482, AC002394, AL109807, and AL050318.
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15	HFXGW52	25	843757	AL022397, AC004087, AC006396, AL031073, AF064860, AP000071, AF003529, AC006070, AL035067, AC006254, AL096770, AL031123, AC000089, AL121578, AL121653, AP000474, AL034402, AJ006345, AC005247, AC006287, AP000966, Z97054, AC007068, AC009891, AL023284, AC007065, AC002067, AL135959, AC005921, AC010197, U95740, AC006210, AC005066, AC003675, AC002449, AC003009, AC007319, AC007376, AC002385, U90095, AJ007973, AC003085, AC005378, AF064863, AC009479, AC005304, AC007546, AC004953, AC007276, AC005023, AL121748, AC007993, AC004242, AL133396, AL121823, AF159056, AL133371, AL117329, AC006033, AL079295, AC005341, Z81369, AL121838, AC008069, AC005180, AC006999, AC009510, U82618, AP000567, AL034548, AL109967, AC008275, AL022150, AC004474, AC007243, AC002541, AC004552, AC006313, AC007326, AR036572, U91328, AC018633, AC004946, AC005772, AL121754, AC005951, AC007541, AP000275, AC005878, AL109922, AC004072, AL133321, AL049647, AP000105, AP000037, AC005083, AC002524, AL023877, AL049873, AL035420, AL008627, AF188024, AC005076, AC002451, and AL008629.
16	HHEQR55	26	799628	L78810, AC006449, AL096701, AL031662, AP000555, AC005899, AC002477, AL139054, AC004967, AP000692, Z85987, AC003104, AC004675, AF165926, AC005231, AC005004, AF088219, Z85986, AL035587, AC007283, AP000252, AC004098, AL035086, U91323, AC006509, AC007216, AL109984, AC002073, AC004859, AF111168, AP000031, AC007371, AP000212, AP000134, AC004099, AL031311, AC006241, AC004000, AC007546, AC004938, AL133448, Z99716, AL133245, AC004263, AC004983, AL049776, AC000353, AF134726, AC005180, AC002470, AC002369, AC004878, AL035659, AL079342, AC005089, AL022165, AC006238, U95742, AC002126, AC005102, AC006211, AC006088, AL034429, Z82190, AC004895, AC005736, AL109801, AC005031, U80017, AC007227, AC002352, AC007308, AC006538, AL049872, AC004841, AC004230, AL035681, AC004656, AL009181, AC004985, AC005844, AC020663, AL096791, AL021707, AC006013, AL022311, AP001053, AC009247, AC005924, AC005011, Z99943, AL020993, AC005919, AL080243, AC005065, AC004922, AC002059, AC008115, AF045555, AC004134, AC002091, and AC004883.
17	HNHNW84	27	843494	AF188024.
18	HKAFH74	44	845530	AI971310, AI937105, AW205490, AW027982, AI638781, AI967925, AI741752, AI871114, AI884846, AW001209, AI986406, AI660973,



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				A07647, AL050149, AF184965, S83440, U62966, U80742, AL050155, AL080148, and AL079340.
19	HCUGE72	29	845785	AI796669, AI554716, AI094845, AI096972, AW105539, AI419333, AA150458, AI887284, AW129537, AI742664, AI147692, AI361320, AI208025, AA868374, AW408004, AA156660, W79942, D51936, AA456475, AA454581, AI096752, AA661639, AA159210, AI139673, AA973820, AA775380, AA837660, AA193428, AW129536, H13480, W74191, AA722061, AI268202, AA160806, AW371152, AA917492, H24656, AI624363, F31640, AA853099, F31383, AA424995, AI803594, AI582388, AI698787, AI242338, H60334, R02760, AI813352, AI376797, Z41319, AA367168, H24655, T98860, AL079470, AA558972, N54091, H59062, AI262885, T99454, R02759, R86215, AI768181, N88601, N84718, AA095359, N84855, AA247964, AA093224, AA096046, N83168, H58760, N83992, AA247827, N84048, N83991, N88782, N89520, AA193451, AA095641, AA096066, N86694, N88518, N87989, N84830, N55698, N83993, N84712, AA471338, N84829, N87898, AB011174, AF045432, AF102850, AF032922, U39066, AF039698, U48696, S78798, AR066487, AF103726, and AJ243486.
19	HCUFM70	45	534502	AA699374.
20	HTEQI22	30	1002360	AA126848, AI660802, AI818138, AI052357, AA314105, AW272820, W91893, AA639648, AI623787, AI339035, AI093450, W07689, AI808940, AI929233, AW136252, AA826484, AI493312, AA258347, AI680254, AA112287, AA809053, T66199, AI087338, AA938320, N36576, AA171466, AA052971, AI198334, W84745, AI445331, AA417087, AA312057, N80566, W84800, AA053448, AW449189, AI186920, AI350841, AI819992, AA113099, AI081825, T87345, AA115583, AI688775, AW452996, W92164, AA026274, W92163, AA135663, AA774265, AA255460, F08938, AA352079, T87446, F12047, AA324094, AA310223, R01422, AA322551, AA258504, AI422532, F13421, W95112, AA384263, AA256858, AA135612, AA368971, AW373743, R01421, N55989, AI014359, AA026273, M85311, F11029, AW272970, M85998, AI570313, R97743, and AA115555.
20	HTEQI22	46	840390	AA126848, AI660802, AI818138, AI052357, AA314105, AW272820, W91893, AA639648, AI623787, AI339035, AI093450, W07689, AI808940, AW136252, AI929233, AA826484, AI493312, AA258347, AI680254, AA112287, AA809053, T66199, AI087338, AA938320, AA171466, AA052971, AI198334, W84745, AI445331, AA417087, AA312057, N80566, W84800, AA053448, AW449189, AI186920, AI350841, AI819992, AI081825, T87345,



				AA115583, AI688775, W92164, AW452996, AA026274, AA774265, AA255460, AA135663, F08938, AA352079, W92163, T87446, F12047, AA310223, R01422, AA322551, AA258504, AI422532, W95112, AA384263, AA256858, AA135612, AA368971, AW373743, R01421, N55989, AI014359, AA026273, M85311, F11029, AW272970, M85998, AI570313, R97743, AA115555, and AA113099.
20	HJBCI01	47	685497	AA126848, AI660802, W91893, AI818138, AI052357, AA310223, AW272820, AA774265, AA314105, AA639648, AI623787, AI339035, AI093450, AI808940, AI929233, AW136252, AA826484, AA258347, AI680254, AA112287, AA809053, W84800, AI493312, W07689, AA258504, AA417087, AI087338, AA171466, AA938320, AI198334, AA052971, W84745, AI445331, T66199, AA312057, N80566, AI186920, AI350841, AA053448, T87345, AI081825, AI688775, AA115583, W92164, AA026274, AA255460, F08938, AA135663, T87446, W92163, R01422, F12047, AA322551, AA352079, AW449189, AI819992, AI422532, W95112, AW452996, AA256858, AA368971, AA135612, AW373743, N55989, R01421, AI014359, AA384263, M85311, AA026273, F11029, AW272970, M85998, AI570313, D80166, D81030, D51799, D51423, R97743, D59619, C14429, D80210, D80240, D80253, D80038, D58283, D59859, D80212, D80188, D80195, D80219, D80227, D80391, D59610, D59889, D80196, D59927, D57483, D80269, D80043, D59502, D80022, D80193, D80366, D59275, D80241, C14331, D80164, D80024, D50979, D80378, D59787, T03269, C75259, D50995, D80045, C14389, C14014, C15076, D59467, D51060, AA305409, D80134, AA115555, AW178893, D81026, D80268, D51250, F13647, D80949, D58253, AW178775, D80168, D81111, D51079, D51022, C14227, AW177440, AW179328, D80522, AW378532, AA305578, AW352158, D59695, AI910186, D80251, C14407, Z21582, AW369651, D80248, AI905856, D52291, AW178762, AW177501, AW177511, AA514188, C14298, AA514186, D80133, D80064, AA285331, D51097, AI557751, AW352117, AW360811, C05695, AW377671, AW176467, AW375405, AW360844, AW378540, AW360834, AW366296, AW352170, AW360817, AW375406, AW378534, AW352171, AW179332, AW377672, AW179023, AW178905, D80132, AW377676, D80439, AW177505, AW179220, D59373, AW360841, D80302, AW178909, AW178906, AW177731, AW178907, AW178754, AW179019, AW179018, AW179024, D80247, AW352172, AW352174, AW179020, D80014, AW177456, AW179329, AW178980, AW177733, AW378528, AW178908,



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21	HDPYE41	31	840022	AA489761, AI028587, AL120563, AA858079, H51121, AC002460, AC005152, AL035684, AC002526, AC005610, AC005076, Z82198, AL049875, AC002385, AL096867, AL031183, AC004938, AC007368, AL034375, AC009294, AP000964, AL035686, AC008080, AC006417, AL049561, AJ010598, AL034417, AC005291, AC002326, AC006961, Z72004, AC007875, AC004632, AL031054, L22023, AL109756, AC005232, AC006256, AL078598, AC005066, AC003046, AL035604, AC007542, AJ239318, AL050305, L10641, AC008275, AC006148, AC004921, AL031285, AL031119, AC005002, AL133246, AC004534, AC008126, AL035106, AC004169, AL078602, AC007370, AC008069, AL049781, AL033377, AL034347, AC007751, AC006043, U69569, and AC003987.
22	HDTII23	32	839393	AA404347, AA680286, AI381446, AI282621, and AA703905.
23	HATCM08	48	778199	AI732223, and AA572709.
23	HTSFV18	49	609939	AW263093, AW138285, AW071991, AI675221, AI554592, AI937646, AI591042, AA812659, AI652785, AI160105, AI360371, AW304967, H49760, AW058610, AI151118, AI198347, AI869732, H21181, AW452721, T56560, R70332, H21180, AI698300, T77409, AI352593, F28930, T60890, T60862, AA351797, AI289791, AW409793, AL036780, AA583873, AW162189, AI635634, AI421523, AA830358, T49776, AL037602, AI097425, AL037582, AW409813, AA001397, AA766258, AW023871, AI961887, AL047675, AW411365, AB009462, AB009463,



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24	HAMFL84	34	837324	AI742901, AW205945, AI092264, AA702443, N31968, AI089509, AI318083, AA991694, AI090124, AI221655, AA515381, AI027137, AA969576, Z38346, W93799, AA569492, AI383617, AI811344, AI801766, AI796743, AW163464, AI439717, AI269696, AI224992, AL134830, AW167410, AI884469, AI621209, AI520785, AI570884, AI475394, AI564247, AW103893, AI539771, AI584140, AI611743, AI619502, AW301505, AI567940, AI886124, AW168795, AI637584, AI802542, AI224027, AI554427, AI684234, AI440239, AL079963, AI690312, AI559296, AW132056, AI475451, AW170635, AI890833, AI926790, AI564719, AI568296, AI954075, AW169790, AI677796, AI696612, AI924971, AW026882, AI687065, F37471, AI889376, AI524671, AI921248, AI590120, AI433976, AI610402, AI362637, AI433157, AI702073, AI590021, AI627988, AI923768, AL119863, AW262565, AI680388, AW023590, AL043293, AI680165, AI569521, AI633125, AI633308, AI857296, AI567846, AL040243, AW150578, AI446373, AI468872, AW051258, AI311926, AI500146, AI859511, AA427700, AI701074, AI476478, AI816947, AI282326, AI274508, AW190042, AI537075, AI922676, AI583308, AI591316, AW088134, AI610115, AW088903, AI537677, AI963216, AA225339, AW081255, AW051107, AI269862, AI648684, AI654750, AI612913, AL039086, AI570384, AI364788, AI620287, AI619749, AL045500, AW079159, AW082040, AW161579, AI934011, AI560099, AI436576, AI921176, AI862139, AI670009, AI689379, AI624206, AW104724, AI499285, AW169653, AI097248, AI498067, AI312542, AI648509, AI888953, AW130776, AW198090, AI682743, AI680498, AI269205, AI569616, AI590227, AW148363, AI679504, AI446684, AI828367, AI498579, AW002342, AI824557, AI702433, AI828731, AI799199, AW149311, AI886753, AW102785, AI612759, AI476109, AI561299, AI815232, AI620284, AI453322, AI868831, AI250663, AI349772, AI633419, AI620003, AI866002, AI335426, AI625384, AI348777, AA835801, AI249962, AI921433, AI573032, AI610645, AI867042, AI916419, AI280661, AI889213, AI431975, AI824648, AI919345, AW071417, AI538085, AL121463, AI569583, AI318280, AI251830, AI539808, AI366549, AI636719, AI539153, AA814407, AL041772, AI336582, AI583065, AI540832, AI249257, AI492528, AI866608, AI954183, AI439745, AI611738,



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				AL110280, I17767, AF095901, U68387, AL117440, A08911, X62580, AF162270, AL137480, AF079763, U68233, I92592, X87582, AL133098, AF185576, AF106827, X92070, AR038854, E08631, L30117, AL137705, AJ006417, AF061573, AF008439, A90832, M30514, U58996, AF132676, AF061836, AL137488, E04233, AF061981, AL133081, AL023657, Y07905, AL137656, U49908, AL121603, AF111849, AL117649, AL137533, AL117432, AF000145, AF067790, AL035067, and AF081197.
27	HOUHD63	37	839414	AI749862, AI950339, AA946724, AI810100, AA418060, AW301664, AA845433, AI982914, W45672, AA807522, AA814824, AI687592, AI472937, AW117375, W45671, AI366028, AI335602, AA417956, AI276538, AA279389, AA291133, AI342816, R56448, N24037, AA991438, U46290, AA815049, H85748, N26756, Z19407, AI080344, AA356371, AI274154, Z28504, AA883996, and H85463.
27	HPJBF63	50	847085	AI749862, AI950339, AA946724, AI810100, AW301664, AA418060, AA845433, AI982914, W45672, AA807522, AA814824, AI687592, AI472937, AW117375, AI366028, W45671, AI335602, AA417956, AI276538, AA279389, AA291133, R56448, N24037, AI342816, AA991438, U46290, AA815049, N26756, H85748, Z19407, AI080344, AA356371, AI274154, Z28504, AA883996, H85463, AC004087, AL008626, Z95116, AC004150, and AC007388.

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Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

5

### Examples

#### Example 1: Isolation of a Selected cDNA Clone From the Deposited Sample

Each cDNA clone in a cited ATCC deposit is contained in a plasmid vector. Table 1 identifies the vectors used to construct the cDNA library from which each clone was isolated. In many cases, the vector used to construct the library is a phage vector from which a plasmid has been excised. The table immediately below correlates the related plasmid for each phage vector used in constructing the cDNA library. For example, where a particular clone is identified in Table 1 as being isolated in the vector "Lambda Zap," the corresponding deposited clone is in "pBluescript."

	<u>Vector Used to Construct Library</u>	<u>Corresponding Deposited</u>
	<u>Plasmid</u>	
	Lambda Zap	pBluescript (pBS)
20	Uni-Zap XR	pBluescript (pBS)
	Zap Express	pBK
	lafmid BA	plafmid BA
	pSport1	pSport1
	pCMVSPORT 2.0	pCMVSPORT 2.0
25	pCMVSPORT 3.0	pCMVSPORT 3.0
	pCR <sup>®</sup> 2.1	pCR <sup>®</sup> 2.1

Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., Nucleic Acids Res. 16:7583-7600 (1988); Altling-Mees, M. A. and Short, J. M., Nucleic Acids Res. 17:9494 (1989)) and pBK (Altling-Mees, M. A. et al., Strategies 5:58-61 (1992)) are commercially available from Stratagene Cloning Systems, Inc., 11011 N. Torrey



Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Both can be transformed into *E. coli* strain XL-1 Blue, also available from Stratagene. pBS comes in 4 forms SK+, SK-, KS+ and KS-. The S and K refers to the orientation of the polylinker to the T7 and T3 primer sequences which flank the polylinker region ("S" is for SacI and "K" is for KpnI which are the first sites on each respective end of the linker). "+" or "-" refer to the orientation of the fl origin of replication ("ori"), such that in one orientation, single stranded rescue initiated from the fl ori generates sense strand DNA and in the other, antisense.

10 Vectors pSport1, pCMVSPORT 2.0 and pCMVSPORT 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, also available from Life Technologies. (See, for instance, Gruber, C. E., et al., Focus 15:59 (1993).) Vector lacmid BA (Bento Soares, Columbia University, NY) contains an ampicillin resistance gene and can be transformed into *E. coli* strain XL-1 Blue. Vector pCR<sup>®</sup>2.1, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, available from Life Technologies. (See, for instance, Clark, J. M., Nuc. Acids Res. 16:9677-9686 (1988) and Mead, D. et al., Bio/Technology 9: (1991).) Preferably, a polynucleotide of the present invention does not comprise the phage vector sequences identified for the particular clone in Table 1, as well as the corresponding plasmid vector sequences designated above.

25 The deposited material in the sample assigned the ATCC Deposit Number cited in Table 1 for any given cDNA clone also may contain one or more additional plasmids, each comprising a cDNA clone different from that given clone. Thus, deposits sharing the same ATCC Deposit Number contain at least a plasmid for each cDNA clone identified in Table 1. Typically, each ATCC deposit sample cited in Table 1 comprises a mixture of approximately equal amounts (by weight) of about 50 plasmid DNAs, each containing a different cDNA clone; but such a deposit sample 30 may include plasmids for more or less than 50 cDNA clones, up to about 500 cDNA clones.



Two approaches can be used to isolate a particular clone from the deposited sample of plasmid DNAs cited for that clone in Table 1. First, a plasmid is directly isolated by screening the clones using a polynucleotide probe corresponding to SEQ ID NO:X.

- 5            Particularly, a specific polynucleotide with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide is labeled, for instance, with  $^{32}\text{P}$ - $\gamma$ -ATP using T4 polynucleotide kinase and purified according to routine methods. (E.g., Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring, NY (1982).)
- 10          The plasmid mixture is transformed into a suitable host, as indicated above (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents cited above. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate.
- 15          These plates are screened using Nylon membranes according to routine methods for bacterial colony screening (e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.

- 20           Alternatively, two primers of 17-20 nucleotides derived from both ends of the SEQ ID NO:X (i.e., within the region of SEQ ID NO:X bounded by the 5' NT and the 3' NT of the clone defined in Table 1) are synthesized and used to amplify the desired cDNA using the deposited cDNA plasmid as a template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25 ul of reaction mixture with 0.5 ug of the above cDNA template. A convenient reaction mixture is
- 25          1.5-5 mM  $\text{MgCl}_2$ , 0.01% (w/v) gelatin, 20 uM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94 degree C for 1 min; annealing at 55 degree C for 1 min; elongation at 72 degree C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and
- 30          the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product.



Several methods are available for the identification of the 5' or 3' non-coding portions of a gene which may not be present in the deposited clone. These methods include but are not limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5' and 3' "RACE" protocols which are well known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript. (Fromont-Racine et al., Nucleic Acids Res. 21(7):1683-1684 (1993).)

Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest is used to PCR amplify the 5' portion of the desired full-length gene. This amplified product may then be sequenced and used to generate the full length gene.

This above method starts with total RNA isolated from the desired source, although poly-A<sup>+</sup> RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the desired gene.

### **Example 2: Isolation of Genomic Clones Corresponding to a Polynucleotide**

A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the cDNA sequence corresponding to SEQ ID NO:X., according to the method described in Example 1. (See also, Sambrook.)



### **Example 3: Tissue Distribution of Polypeptide**

Tissue distribution of mRNA expression of polynucleotides of the present invention is determined using protocols for Northern blot analysis, described by, among others, Sambrook et al. For example, a cDNA probe produced by the method described in Example 1 is labeled with P<sup>32</sup> using the rediprime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various human tissues for mRNA expression.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) (Clontech) are examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70 degree C overnight, and the films developed according to standard procedures.

### **Example 4: Chromosomal Mapping of the Polynucleotides**

An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:X. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions : 30 seconds, 95 degree C; 1 minute, 56 degree C; 1 minute, 70 degree C. This cycle is repeated 32 times followed by one 5 minute cycle at 70 degree C. Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions is analyzed on either 8% polyacrylamide gels or 3.5 % agarose gels. Chromosome mapping is determined by the presence of an approximately 100 bp PCR fragment in the particular somatic cell hybrid.

### **Example 5: Bacterial Expression of a Polypeptide**



A polynucleotide encoding a polypeptide of the present invention is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites, such as BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp<sup>r</sup>), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites.

The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan<sup>r</sup>). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.<sup>600</sup>) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.

Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl by stirring for 3-4 hours at 4 degree C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with



high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., *supra*).

Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then  
 5 washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-  
 10 NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250  
 15 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4 degree C or frozen at -80 degree C.

In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements operatively linked to a polynucleotide of the present invention, called pHE4a. (ATCC  
 20 Accession Number 209645, deposited on February 25, 1998.) This vector contains:  
 1) a neomycinphosphotransferase gene as a selection marker, 2) an E. coli origin of replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (*lacIq*). The origin of replication (*oriC*) is derived from pUC19 (LTI, Gaithersburg, MD). The  
 25 promoter sequence and operator sequences are made synthetically.

DNA can be inserted into the pHEa by restricting the vector with NdeI and XbaI, BamHI, XhoI, or Asp718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA insert is generated according to the PCR protocol described in Example 1, using PCR  
 30 primers having restriction sites for NdeI (5' primer) and XbaI, BamHI, XhoI, or Asp718 (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated according to standard protocols.



The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system.

### **Example 6: Purification of a Polypeptide from an Inclusion Body**

The following alternative method can be used to purify a polypeptide expressed in *E coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10 degree C.

Upon completion of the production phase of the *E. coli* fermentation, the cell culture is cooled to 4-10 degree C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

15           The cells are then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

20           The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4 degree C overnight to allow further GuHCl extraction.

Following high speed centrifugation (30,000 xg) to remove insoluble particles,  
25 the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with  
20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA  
by vigorous stirring. The refolded diluted protein solution is kept at 4 degree C  
without mixing for 12 hours prior to further purification steps.

To clarify the refolded polypeptide solution, a previously prepared tangential  
30 filtration unit equipped with 0.16  $\mu$ m membrane filter with appropriate surface area  
(e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The  
filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive



Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

5 Fractions containing the polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40  
10 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant  $A_{280}$  monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

15 The resultant polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Commassie blue stained 16% SDS-PAGE gel when 5 ug of purified protein is loaded. The purified protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL  
20 assays.

### **Example 7: Cloning and Expression of a Polypeptide in a Baculovirus**

#### **Expression System**

In this example, the plasmid shuttle vector pA2 is used to insert a  
25 polynucleotide into a baculovirus to express a polypeptide. This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the  
30 plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak *Drosophila* promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral



sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., *Virology* 170:31-39 (1989).

Specifically, the cDNA sequence contained in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence identified in Table 1, is amplified using the PCR protocol described in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the pA2 vector does not need a second signal peptide. Alternatively, the vector can be modified (pA2 GP) to include a baculovirus leader sequence, using the standard methods described in Summers et al., "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, Ca.).

The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.



Five ug of a plasmid containing the polynucleotide is co-transfected with 1.0 ug of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987). One ug of BaculoGold™ virus DNA and 5 ug of the plasmid are mixed in a sterile well of a microtiter plate containing 50 ul of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 ul Lipofectin plus 90 ul Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27 degrees C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27 degrees C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 ul of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4 degree C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 uCi of <sup>35</sup>S-methionine and 5 uCi <sup>35</sup>S-cysteine (available from Amersham) are added.



The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of  
 5 purified protein may be used to determine the amino terminal sequence of the produced protein.

### **Example 8: Expression of a Polypeptide in Mammalian Cells**

The polypeptide of the present invention can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which  
 10 mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long  
 15 terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden),  
 20 pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109), pCMVSPORT 2.0, and pCMVSPORT 3.0. Mammalian host cells that could be used include, human HeLa, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

25 Alternatively, the polypeptide can be expressed in stable cell lines containing the polynucleotide integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the  
 30 encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of



interest. (See, e.g., Alt, F. W., et al., J. Biol. Chem. 253:1357-1370 (1978); Hamlin, J. L. and Ma, C., Biochem. et Biophys. Acta, 1097:107-143 (1990); Page, M. J. and Sydenham, M. A., Biotechnology 9:64-68 (1991).) Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., Biochem J. 227:277-279 (1991); Bebbington et al., Bio/Technology 10:169-175 (1992). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

Derivatives of the plasmid pSV2-dhfr (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No. 209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., Molecular and Cellular Biology, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., Cell 41:521-530 (1985).) Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

Specifically, the plasmid pC6, for example, is digested with appropriate restriction enzymes and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

A polynucleotide of the present invention is amplified according to the protocol outlined in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the vector does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector



are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for  
 5 transfection. Five  $\mu\text{g}$  of the expression plasmid pC6 a pC4 is cotransfected with 0.5  
 $\mu\text{g}$  of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-  
 neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an  
 enzyme that confers resistance to a group of antibiotics including G418. The cells are  
 seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the  
 10 cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in  
 alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1  
 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in  
 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50  
 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest  
 15 concentrations of methotrexate are then transferred to new 6-well plates containing  
 even higher concentrations of methotrexate (1  $\mu\text{M}$ , 2  $\mu\text{M}$ , 5  $\mu\text{M}$ , 10 mM, 20 mM).  
 The same procedure is repeated until clones are obtained which grow at a  
 concentration of 100 - 200  $\mu\text{M}$ . Expression of the desired gene product is analyzed,  
 for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

20

### **Example 9: Protein Fusions**

The polypeptides of the present invention are preferably fused to other  
 proteins. These fusion proteins can be used for a variety of applications. For  
 example, fusion of the present polypeptides to His-tag, HA-tag, protein A, IgG  
 25 domains, and maltose binding protein facilitates purification. (See Example 5; see  
 also EP A 394,827; Traunecker, et al., Nature 331:84-86 (1988).) Similarly, fusion to  
 IgG-1, IgG-3, and albumin increases the halflife time in vivo. Nuclear localization  
 signals fused to the polypeptides of the present invention can target the protein to a  
 specific subcellular localization, while covalent heterodimer or homodimers can  
 30 increase or decrease the activity of a fusion protein. Fusion proteins can also create  
 chimeric molecules having more than one function. Finally, fusion proteins can  
 increase solubility and/or stability of the fused protein compared to the non-fused



protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule, or the protocol described in Example 5.

Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

For example, if pC4 (Accession No. 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and a polynucleotide of the present invention, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

If the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

Human IgG Fc region:

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GGGATCCGGAGCCCAAATCTTCTGACAAAACCTCACACATGCCCACCGTGC
CCAGCACCTGAATTCGAGGGTGCACCGTCAGTCTTCCTCTTCCCCCAAAA
CCCAAGGACACCCTCATGATCTCCCGGACTCCTGAGGTCACATGCGTGGT
GGTGGACGTAAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGG
ACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTA
CAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACT
GGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCA
ACCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC
CACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAG
GTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGACATCGCCGT
GGAGTGGGAGAGCAATGGGCAGCCGGAACAACACTACAAGACCACGCCT
CCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTG

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GACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCA  
 TGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGG  
 GTAAATGAGTGCGACGGCCGCGACTCTAGAGGAT (SEQ ID NO:1)

#### 5 **Example 10: Production of an Antibody from a Polypeptide**

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing a polypeptide of the present invention is administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred  
 10 method, a preparation of the secreted protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or protein binding fragments thereof). Such monoclonal  
 15 antibodies can be prepared using hybridoma technology. (Köhler et al., Nature 256:495 (1975); Köhler et al., Eur. J. Immunol. 6:511 (1976); Köhler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981).) In general, such procedures involve immunizing an animal (preferably a mouse) with polypeptide or, more  
 20 preferably, with a secreted polypeptide-expressing cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56 degrees C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 ug/ml of streptomycin.

25 The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as  
 30 described by Wands et al. (Gastroenterology 80:225-232 (1981).) The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide.



Alternatively, additional antibodies capable of binding to the polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the protein-specific antibody can be blocked by the polypeptide. Such antibodies comprise anti-idiotypic antibodies to the protein-specific antibody and can be used to immunize an animal to induce formation of further protein-specific antibodies.

It will be appreciated that Fab and F(ab')<sub>2</sub> and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). Alternatively, secreted protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

For in vivo use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

### **Example 11: Production Of Secreted Protein For High-Throughput Screening**

#### **Assays**

The following protocol produces a supernatant containing a polypeptide to be tested. This supernatant can then be used in the Screening Assays described herein.



First, dilute Poly-D-Lysine (644 587 Boehringer-Mannheim) stock solution (1mg/ml in PBS) 1:20 in PBS (w/o calcium or magnesium 17-516F Biowhittaker) for a working solution of 50ug/ml. Add 200 ul of this solution to each well (24 well plates) and incubate at RT for 20 minutes. Be sure to distribute the solution over each well (note: a 12-channel pipetter may be used with tips on every other channel). Aspirate off the Poly-D-Lysine solution and rinse with 1ml PBS (Phosphate Buffered Saline). The PBS should remain in the well until just prior to plating the cells and plates may be poly-lysine coated in advance for up to two weeks.

- 10 Plate 293T cells (do not carry cells past P+20) at  $2 \times 10^5$  cells/well in .5ml DMEM(Dulbecco's Modified Eagle Medium)(with 4.5 G/L glucose and L-glutamine (12-604F Biowhittaker))/10% heat inactivated FBS(14-503F Biowhittaker)/1x Penstrep(17-602E Biowhittaker). Let the cells grow overnight.

- 15 The next day, mix together in a sterile solution basin: 300 ul Lipofectamine (18324-012 Gibco/BRL) and 5ml Optimem I (31985070 Gibco/BRL)/96-well plate. With a small volume multi-channel pipetter, aliquot approximately 2ug of an expression vector containing a polynucleotide insert, produced by the methods described in Examples 8 or 9, into an appropriately labeled 96-well round bottom plate. With a multi-channel pipetter, add 50ul of the Lipofectamine/Optimem I mixture to each well. Pipette up and down gently to mix. Incubate at RT 15-45 minutes. After about 20 minutes, use a multi-channel pipetter to add 150ul Optimem I to each well. As a control, one plate of vector DNA lacking an insert should be transfected with each set of transfections.

- 25 Preferably, the transfection should be performed by tag-teaming the following tasks. By tag-teaming, hands on time is cut in half, and the cells do not spend too much time on PBS. First, person A aspirates off the media from four 24-well plates of cells, and then person B rinses each well with .5-1ml PBS. Person A then aspirates off PBS rinse, and person B, using a 12-channel pipetter with tips on every other channel, adds the 200ul of DNA/Lipofectamine/Optimem I complex to the odd wells first, then to the even wells, to each row on the 24-well plates. Incubate at 37 degrees C for 6 hours.

While cells are incubating, prepare appropriate media, either 1%BSA in DMEM with 1x penstrep, or CHO-5 media (116.6 mg/L of CaCl<sub>2</sub> (anhyd); 0.00130



mg/L  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ; 0.050 mg/L of  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ; 0.417 mg/L of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ; 311.80 mg/L of KCl; 28.64 mg/L of  $\text{MgCl}_2$ ; 48.84 mg/L of  $\text{MgSO}_4$ ; 6995.50 mg/L of NaCl; 2400.0 mg/L of  $\text{NaHCO}_3$ ; 62.50 mg/L of  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ; 71.02 mg/L of  $\text{Na}_2\text{HPO}_4$ ; .4320 mg/L of  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ; .002 mg/L of Arachidonic Acid ; 1.022 mg/L of Cholesterol; .070 mg/L of DL-alpha-Tocopherol-Acetate; 0.0520 mg/L of Linoleic Acid; 0.010 mg/L of Linolenic Acid; 0.010 mg/L of Myristic Acid; 0.010 mg/L of Oleic Acid; 0.010 mg/L of Palmitic Acid; 0.010 mg/L of Palmitic Acid; 100 mg/L of Pluronic F-68; 0.010 mg/L of Stearic Acid; 2.20 mg/L of Tween 80; 4551 mg/L of D-Glucose; 130.85 mg/ml of L- Alanine; 147.50 mg/ml of L-Arginine-HCL; 7.50 mg/ml of L-Asparagine- $\text{H}_2\text{O}$ ; 6.65 mg/ml of L-Aspartic Acid; 29.56 mg/ml of L-Cystine-2HCL- $\text{H}_2\text{O}$ ; 31.29 mg/ml of L-Cystine-2HCL; 7.35 mg/ml of L-Glutamic Acid; 365.0 mg/ml of L-Glutamine; 18.75 mg/ml of Glycine; 52.48 mg/ml of L-Histidine-HCL- $\text{H}_2\text{O}$ ; 106.97 mg/ml of L-Isoleucine; 111.45 mg/ml of L-Leucine; 163.75 mg/ml of L-Lysine HCL; 32.34 mg/ml of L-Methionine; 68.48 mg/ml of L-Phenylalanine; 40.0 mg/ml of L-Proline; 26.25 mg/ml of L-Serine; 101.05 mg/ml of L-Threonine; 19.22 mg/ml of L-Tryptophan; 91.79 mg/ml of L-Tyrosine-2Na-2 $\text{H}_2\text{O}$ ; 99.65 mg/ml of L-Valine; 0.0035 mg/L of Biotin; 3.24 mg/L of D-Ca Pantothenate; 11.78 mg/L of Choline Chloride; 4.65 mg/L of Folic Acid; 15.60 mg/L of i-Inositol; 3.02 mg/L of Niacinamide; 3.00 mg/L of Pyridoxal HCL; 0.031 mg/L of Pyridoxine HCL; 0.319 mg/L of Riboflavin; 3.17 mg/L of Thiamine HCL; 0.365 mg/L of Thymidine; and 0.680 mg/L of Vitamin  $\text{B}_{12}$ ; 25 mM of HEPES Buffer; 2.39 mg/L of Na Hypoxanthine; 0.105 mg/L of Lipoic Acid; 0.081 mg/L of Sodium Putrescine-2HCL; 55.0 mg/L of Sodium Pyruvate; 0.0067 mg/L of Sodium Selenite; 20uM of Ethanolamine; 0.122 mg/L of Ferric Citrate; 41.70 mg/L of Methyl-B-Cyclodextrin complexed with Linoleic Acid; 33.33 mg/L of Methyl-B-Cyclodextrin complexed with Oleic Acid; and 10 mg/L of Methyl-B-Cyclodextrin complexed with Retinal) with 2mm glutamine and 1x penstrep. (BSA (81-068-3 Bayer) 100gm dissolved in 1L DMEM for a 10% BSA stock solution). Filter the media and collect 50 ul for endotoxin assay in 15ml polystyrene conical.

The transfection reaction is terminated, preferably by tag-teaming, at the end of the incubation period. Person A aspirates off the transfection media, while person



B adds 1.5ml appropriate media to each well. Incubate at 37 degrees C for 45 or 72 hours depending on the media used: 1%BSA for 45 hours or CHO-5 for 72 hours.

On day four, using a 300ul multichannel pipetter, aliquot 600ul in one 1ml deep well plate and the remaining supernatant into a 2ml deep well. The supernatants  
5 from each well can then be used in the assays described in Examples 13-20.

It is specifically understood that when activity is obtained in any of the assays described below using a supernatant, the activity originates from either the polypeptide directly (e.g., as a secreted protein) or by the polypeptide inducing expression of other proteins, which are then secreted into the supernatant. Thus, the  
10 invention further provides a method of identifying the protein in the supernatant characterized by an activity in a particular assay.

#### **Example 12: Construction of GAS Reporter Construct**

One signal transduction pathway involved in the differentiation and  
15 proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the Jaks-STATs pathway bind to gamma activation site "GAS" elements or interferon-sensitive responsive element ("ISRE"), located in the promoter of many genes. The binding of a protein to these elements alter the expression of the associated gene.

GAS and ISRE elements are recognized by a class of transcription factors  
20 called Signal Transducers and Activators of Transcription, or "STATs." There are six members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in many cell types though it has been found in T helper class I, cells after treatment with IL-12. Stat5 was originally called mammary growth factor, but has been found at  
25 higher concentrations in other cells including myeloid cells. It can be activated in tissue culture cells by many cytokines.

The STATs are activated to translocate from the cytoplasm to the nucleus upon tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2,  
30 Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are generally catalytically inactive in resting cells.



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To construct a synthetic GAS containing promoter element, which is used in the Biological Assays described in Examples 13-14, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5' primer contains four tandem copies of the GAS binding site found in the IRF1 promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothman et al., Immunity 1:457-468 (1994).), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18bp of sequence complementary to the SV40 early promoter sequence and is flanked with an XhoI site. The sequence of the 5' primer is:

10 5':GCGCCTCGAGATTTCCTCCGAAATCTAGATTTCCTCCGAAATGATTTCCTCCGAAATGATTTCCTCCGAAATATCTGCCATCTCAATTAG:3' (SEQ ID NO:3)

The downstream primer is complementary to the SV40 promoter and is flanked with a Hind III site: 5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:4)

15 PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI/Hind III and subcloned into BLSK2-. (Stratagene.) Sequencing with forward and reverse primers confirms that the insert contains the following sequence:

20 5':CTCGAGATTTCCTCCGAAATCTAGATTTCCTCCGAAATGATTTCCTCCGAAATGATTTCCTCCGAAATATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCC  
TCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCT  
25 AGGCTTTTGCAAAAAGCTT:3' (SEQ ID NO:5)

With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP." Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.



The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using HindIII and XhoI, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter element, to create the GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Thus, in order to generate mammalian stable cell lines expressing the GAS-SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector using SalI and NotI, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding as described in Examples 13-14.

Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NFK-B and EGR promoter sequences are described in Examples 15 and 16. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, IL-2/NFAT, or NF-KB/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HELA (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

### **Example 13: High-Throughput Screening Assay for T-cell Activity.**

The following protocol is used to assess T-cell activity by identifying factors, and determining whether supernate containing a polypeptide of the invention proliferates and/or differentiates T-cells. T-cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway.

The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152), although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC Accession No. CRL-1582) cells can also be used.



Jurkat T-cells are lymphoblastic CD4<sup>+</sup> Th1 helper cells. In order to generate stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo vector using DMRIE-C (Life Technologies)(transfection procedure described below). The transfected cells are seeded to a density of approximately 20,000 cells per well and transfectants resistant to 1 mg/ml gentamicin selected. Resistant colonies are expanded and then tested for their response to increasing concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

Specifically, the following protocol will yield sufficient cells for 75 wells containing 200 ul of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient cells for multiple 96 well plates. Jurkat cells are maintained in RPMI + 10% serum with 1% Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life Technologies) with 10 ug of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50 ul of DMRIE-C and incubate at room temperature for 15-45 mins.

During the incubation period, count cell concentration, spin down the required number of cells ( $10^7$  per transfection), and resuspend in OPTI-MEM to a final concentration of  $10^7$  cells/ml. Then add 1ml of  $1 \times 10^7$  cells in OPTI-MEM to T25 flask and incubate at 37 degrees C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

The Jurkat:GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Gentamicin, and 1% Pen-Strep. These cells are treated with supernatants containing polypeptides of the invention and/or induced polypeptides of the invention as produced by the protocol described in Example 11.

On the day of treatment with the supernatant, the cells should be washed and resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of supernatants being screened. For one 96 well plate, approximately 10 million cells (for 10 plates, 100 million cells) are required.

Transfer the cells to a triangular reservoir boat, in order to dispense the cells into a 96 well dish, using a 12 channel pipette. Using a 12 channel pipette, transfer 200 ul of cells into each well (therefore adding 100, 000 cells per well).



After all the plates have been seeded, 50  $\mu$ l of the supernatants are transferred directly from the 96 well plate containing the supernatants into each well using a 12 channel pipette. In addition, a dose of exogenous interferon gamma (0.1, 1.0, 10 ng) is added to wells H9, H10, and H11 to serve as additional positive controls for the assay.

The 96 well dishes containing Jurkat cells treated with supernatants are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35  $\mu$ l samples from each well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophane covers) and stored at  $-20^{\circ}\text{C}$  until SEAP assays are performed according to Example 17. The plates containing the remaining treated cells are placed at  $4^{\circ}\text{C}$  and serve as a source of material for repeating the assay on a specific well if desired.

As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

The above protocol may be used in the generation of both transient, as well as, stable transfected cells, which would be apparent to those of skill in the art.

#### **Example 14: High-Throughput Screening Assay Identifying Myeloid Activity**

The following protocol is used to assess myeloid activity by determining whether polypeptides of the invention proliferates and/or differentiates myeloid cells. Myeloid cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

To transiently transfect U937 cells with the GAS/SEAP/Neo construct produced in Example 12, a DEAE-Dextran method (Kharbanda et. al., 1994, Cell Growth & Differentiation, 5:259-265) is used. First, harvest  $2 \times 10^7$  U937 cells and wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.



Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing 0.5 mg/ml DEAE-Dextran, 8 ug GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM KCl, 375 uM Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O, 1 mM MgCl<sub>2</sub>, and 675 uM CaCl<sub>2</sub>. Incubate at 37 degrees C for 45 min.

- 5 Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37 degrees C for 36 hr.

The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400 ug/ml G418 for couple of passages.

- 10 These cells are tested by harvesting  $1 \times 10^8$  cells (this is enough for ten 96-well plates assay) and wash with PBS. Suspend the cells in 200 ml above described growth medium, with a final density of  $5 \times 10^5$  cells/ml. Plate 200 ul cells per well in the 96-well plate (or  $1 \times 10^5$  cells/well).

- 15 Add 50 ul of the supernatant prepared by the protocol described in Example 11. Incubate at 37 degrees C for 48 to 72 hr. As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate U937 cells. Over 30 fold induction is typically observed in the positive control wells. SEAP assay the supernatant according to the protocol described in Example 17.

#### 20 **Example 15: High-Throughput Screening Assay Identifying Neuronal Activity.**

- When cells undergo differentiation and proliferation, a group of genes are activated through many different signal transduction pathways. One of these genes, EGR1 (early growth response gene 1), is induced in various tissues and cell types upon activation. The promoter of EGR1 is responsible for such induction. Using the  
25 EGR1 promoter linked to reporter molecules, activation of cells can be assessed.

- Particularly, the following protocol is used to assess neuronal activity in PC12 cell lines. PC12 cells (rat pheochromocytoma cells) are known to proliferate and/or differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor).  
30 The EGR1 gene expression is activated during this treatment. Thus, by stably transfecting PC12 cells with a construct containing an EGR promoter linked to SEAP reporter, activation of PC12 cells can be assessed.



5' GCGCTCGAGGGATGACAGCGATAGAACCCCGG -3' (SEQ ID NO:6)  
5' GCGAAGCTTCGCGACTCCCCGGATCCGCCTC-3' (SEQ ID NO:7)

To prepare 96 well-plates for cell culture, two mls of a coating solution (1:30 dilution of collagen type I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter sterilized)) is added per one 10 cm plate or 50 ml per well of the 96-well plate, and allowed to air dry for 2 hr.

Transfect the EGR/SEAP/Neo construct into PC12 using the Lipofectamine protocol described in Example 11. EGR-SEAP/PC12 stable cells are obtained by growing the cells in 300 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 300 ug/ml G418 for couple of passages.

The next morning, remove the medium and wash the cells with PBS. Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count



the cell number and add more low serum medium to reach final cell density as  $5 \times 10^5$  cells/ml.

Add 200  $\mu$ l of the cell suspension to each well of 96-well plate (equivalent to  $1 \times 10^5$  cells/well). Add 50  $\mu$ l supernatant produced by Example 11, 37°C for 48 to 72 hr. As a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/ $\mu$ l of Neuronal Growth Factor (NGF). Over fifty-fold induction of SEAP is typically seen in the positive control wells. SEAP assay the supernatant according to Example 17.

#### 10 **Example 16: High-Throughput Screening Assay for T-cell Activity**

NF-KB (Nuclear Factor KB) is a transcription factor activated by a wide variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, NF-KB regulates the expression of genes involved in immune cell activation, control of apoptosis (NF- KB appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

In non-stimulated conditions, NF- KB is retained in the cytoplasm with I-KB (Inhibitor KB). However, upon stimulation, I- KB is phosphorylated and degraded, causing NF- KB to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF- KB include IL-2, IL-6, GM-CSF, ICAM-1 and class 1 MHC.

Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF-KB promoter element are used to screen the supernatants produced in Example 11. Activators or inhibitors of NF-KB would be useful in treating diseases. For example, inhibitors of NF-KB could be used to treat those diseases related to the acute or chronic activation of NF-KB, such as rheumatoid arthritis.

To construct a vector containing the NF-KB promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the NF-KB binding site (GGGGACTTTCCC) (SEQ ID NO:8), 18 bp of sequence



complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an XhoI site:

5':GCGGCCTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGAC  
TTTCCATCCTGCCATCTCAATTAG:3' (SEQ ID NO:9)

- 5 The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a Hind III site:

5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:4)

- PCR amplification is performed using the SV40 promoter template present in the pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is  
10 digested with XhoI and Hind III and subcloned into BLSK2-. (Stratagene)  
Sequencing with the T7 and T3 primers confirms the insert contains the following sequence:

- 5':CTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGACTTTCC  
15 ATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCC  
ATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGA  
CTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTA  
TTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAA  
GCTT:3' (SEQ ID NO:10)

20

Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this NF-KB/SV40 fragment using XhoI and HindIII. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

- 25 In order to generate stable mammalian cell lines, the NF-KB/SV40/SEAP cassette is removed from the above NF-KB/SEAP vector using restriction enzymes SalI and NotI, and inserted into a vector containing neomycin resistance. Particularly, the NF-KB/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-1 with SalI and NotI.

- 30 Once NF-KB/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 13. Similarly, the method for assaying supernatants with these stable Jurkat T-cells is also described



in Example 13. As a positive control, exogenous TNF alpha (0.1, 1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.

#### **Example 17: Assay for SEAP Activity**

5 As a reporter molecule for the assays described in Examples 13-16, SEAP activity is assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-light Kit supplies the Dilution, Assay, and Reaction Buffers used below.

10 Prime a dispenser with the 2.5x Dilution Buffer and dispense 15 ul of 2.5x dilution buffer into Optiplates containing 35 ul of a supernatant. Seal the plates with a plastic sealer and incubate at 65 degree C for 30 min. Separate the Optiplates to avoid uneven heating.

15 Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50 ml Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the table below). Add 50 ul Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on luminometer, one should treat 5 plates at each time and start the second set 10 minutes later.

20 Read the relative light unit in the luminometer. Set H12 as blank, and print the results. An increase in chemiluminescence indicates reporter activity.

#### **Reaction Buffer Formulation:**

# of plates	Rxn buffer diluent (ml)	CSPD (ml)
10	60	3
11	65	3.25
12	70	3.5
13	75	3.75
14	80	4
15	85	4.25
16	90	4.5
17	95	4.75
18	100	5
19	105	5.25
20	110	5.5
21	115	5.75
22	120	6
23	125	6.25
24	130	6.5



25	135	6.75
26	140	7
27	145	7.25
28	150	7.5
29	155	7.75
30	160	8
31	165	8.25
32	170	8.5
33	175	8.75
34	180	9
35	185	9.25
36	190	9.5
37	195	9.75
38	200	10
39	205	10.25
40	210	10.5
41	215	10.75
42	220	11
43	225	11.25
44	230	11.5
45	235	11.75
46	240	12
47	245	12.25
48	250	12.5
49	255	12.75
50	260	13

### **Example 18: High-Throughput Screening Assay Identifying Changes in Small Molecule Concentration and Membrane Permeability**

Binding of a ligand to a receptor is known to alter intracellular levels of small molecules, such as calcium, potassium, sodium, and pH, as well as alter membrane potential. These alterations can be measured in an assay to identify supernatants which bind to receptors of a particular cell. Although the following protocol describes an assay for calcium, this protocol can easily be modified to detect changes in potassium, sodium, pH, membrane potential, or any other small molecule which is detectable by a fluorescent probe.

The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-4 (Molecular Probes, Inc.; catalog no. F-14202), used here.

For adherent cells, seed the cells at 10,000 -20,000 cells/well in a Co-star black 96-well plate with clear bottom. The plate is incubated in a CO<sub>2</sub> incubator for



20 hours. The adherent cells are washed two times in Biotek washer with 200 ul of HBSS (Hank's Balanced Salt Solution) leaving 100 ul of buffer after the final wash.

A stock solution of 1 mg/ml fluo-4 is made in 10% pluronic acid DMSO. To load the cells with fluo-4, 50 ul of 12 ug/ml fluo-4 is added to each well. The plate is incubated at 37 degrees C in a CO<sub>2</sub> incubator for 60 min. The plate is washed four times in the Biotek washer with HBSS leaving 100 ul of buffer.

For non-adherent cells, the cells are spun down from culture media. Cells are re-suspended to  $2-5 \times 10^6$  cells/ml with HBSS in a 50-ml conical tube. 4 ul of 1 mg/ml fluo-4 solution in 10% pluronic acid DMSO is added to each ml of cell suspension.

The tube is then placed in a 37 degrees C water bath for 30-60 min. The cells are washed twice with HBSS, resuspended to  $1 \times 10^6$  cells/ml, and dispensed into a microplate, 100 ul/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is then washed once in Denley CellWash with 200 ul, followed by an aspiration step to 100 ul final volume.

For a non-cell based assay, each well contains a fluorescent molecule, such as fluo-4. The supernatant is added to the well, and a change in fluorescence is detected.

To measure the fluorescence of intracellular calcium, the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4 second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm; and (6) Sample addition is 50 ul. Increased emission at 530 nm indicates an extracellular signaling event which has resulted in an increase in the intracellular Ca<sup>++</sup> concentration.

#### **Example 19: High-Throughput Screening Assay Identifying Tyrosine Kinase Activity**

The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase (RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is



unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., src, yes, lck, lyn, fyn) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

Because of the wide range of known factors capable of stimulating tyrosine kinase activity, the identification of novel human secreted proteins capable of activating tyrosine kinase signal transduction pathways are of interest. Therefore, the following protocol is designed to identify those novel human secreted proteins capable of activating the tyrosine kinase signal transduction pathways.

Seed target cells (e.g., primary keratinocytes) at a density of approximately 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or 10% Matrigel purchased from Becton Dickinson (Bedford, MA), or calf serum, rinsed with PBS and stored at 4 degree C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamarBlue as described by the manufacturer Alamar Biosciences, Inc. (Sacramento, CA) after 48 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford, MA) are used to cover the Loprodyne Silent Screen Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced by incubation in serum-free basal medium for 24 hr. After 5-20 minutes treatment with EGF (60ng/ml) or 50 ul of the supernatant produced in



Example 11, the medium was removed and 100 ml of extraction buffer ((20 mM HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na<sub>3</sub>VO<sub>4</sub>, 2 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> and a cocktail of protease inhibitors (# 1836170) obtained from Boehringer Mannheim (Indianapolis, IN) is added to each well and the plate is  
 5 shaken on a rotating shaker for 5 minutes at 4 degrees C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 mm membrane bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by centrifugation, the content of each well, after  
 10 detergent solubilization for 5 minutes, is removed and centrifuged for 15 minutes at 4 degrees C at 16,000 x g.

Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

15 Generally, the tyrosine kinase activity of a supernatant is evaluated by determining its ability to phosphorylate a tyrosine residue on a specific substrate (a biotinylated peptide). Biotinylated peptides that can be used for this purpose include PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for  
 20 a range of tyrosine kinases and are available from Boehringer Mannheim.

The tyrosine kinase reaction is set up by adding the following components in order. First, add 10ul of 5uM Biotinylated Peptide, then 10ul ATP/Mg<sub>2</sub><sup>+</sup> (5mM ATP/50mM MgCl<sub>2</sub>), then 10ul of 5x Assay Buffer (40mM imidazole hydrochloride, pH7.3, 40 mM beta-glycerophosphate, 1mM EGTA, 100mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>,  
 25 0.5 mg/ml BSA), then 5ul of Sodium Vanadate(1mM), and then 5ul of water. Mix the components gently and preincubate the reaction mix at 30 degrees C for 2 min. Initial the reaction by adding 10ul of the control enzyme or the filtered supernatant.

The tyrosine kinase assay reaction is then terminated by adding 10 ul of 120mM EDTA and place the reactions on ice.

30 Tyrosine kinase activity is determined by transferring 50 ul aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37 degrees C for 20



5

10

### Activity

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cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6ng/well) or 50 ul of the supernatants obtained in Example 11 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

- 5           After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (1ug/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard
- 10           procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation.

15           **Example 21: Method of Determining Alterations in a Gene Corresponding to a Polynucleotide**

- RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is be isolated. cDNA is then generated from these RNA samples using protocols known in the art. (See, Sambrook.) The cDNA
- 20           is then used as a template for PCR, employing primers surrounding regions of interest in SEQ ID NO:X. Suggested PCR conditions consist of 35 cycles at 95 degrees C for 30 seconds; 60-120 seconds at 52-58 degrees C; and 60-120 seconds at 70 degrees C, using buffer solutions described in Sidransky et al., Science 252:706 (1991).

- PCR products are then sequenced using primers labeled at their 5' end with T4
- 25           polynucleotide kinase, employing SequiTherm Polymerase. (Epicentre Technologies). The intron-exon borders of selected exons is also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations is then cloned and sequenced to validate the results of the direct sequencing.

- 30           PCR products is cloned into T-tailed vectors as described in Holton et al., Nucleic Acids Research, 19:1156 (1991) and sequenced with T7 polymerase (United



States Biochemical). Affected individuals are identified by mutations not present in unaffected individuals.

Genomic rearrangements are also observed as a method of determining alterations in a gene corresponding to a polynucleotide. Genomic clones isolated according to Example 2 are nick-translated with digoxigenin deoxy-uridine 5'-triphosphate (Boehringer Mannheim), and FISH performed as described in Johnson et al., *Methods Cell Biol.* 35:73-99 (1991). Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA for specific hybridization to the corresponding genomic locus.

Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson et al., *Genet. Anal. Tech. Appl.*, 8:75 (1991).) Image collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region hybridized by the probe are identified as insertions, deletions, and translocations. These alterations are used as a diagnostic marker for an associated disease.

#### **Example 22: Method of Detecting Abnormal Levels of a Polypeptide in a Biological Sample**

A polypeptide of the present invention can be detected in a biological sample, and if an increased or decreased level of the polypeptide is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect polypeptides in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described in



Example 10. The wells are blocked so that non-specific binding of the polypeptide to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample containing the polypeptide. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbounded polypeptide.

Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

Add 75 ul of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the polypeptide in the sample using the standard curve.

### **Example 23: Formulation**

The invention also provides methods of treatment and/or prevention diseases, disorders, and/or conditions (such as, for example, any one or more of the diseases or disorders disclosed herein) by administration to a subject of an effective amount of a Therapeutic. By therapeutic is meant a polynucleotides or polypeptides of the invention (including fragments and variants), agonists or antagonists thereof, and/or antibodies thereto, in combination with a pharmaceutically acceptable carrier type (e.g., a sterile carrier).

The Therapeutic will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the Therapeutic alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.



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(for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al.,  
 5 Biopolymers 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (Langer et al., J. Biomed. Mater. Res. 15:167-277 (1981), and Langer, Chem. Tech. 12:98-105 (1982)), ethylene vinyl acetate (Langer et al., Id.) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988).

Sustained-release Therapeutics also include liposomally entrapped  
 10 Therapeutics of the invention (*see* generally, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317 -327 and 353-365 (1989)). Liposomes containing the Therapeutic are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang  
 15 et al., Proc. Natl. Acad. Sci.(USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the  
 20 optimal Therapeutic.

In yet an additional embodiment, the Therapeutics of the invention are delivered by way of a pump (*see* Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)).

25 Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

For parenteral administration, in one embodiment, the Therapeutic is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically  
 30 acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation.



For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to the Therapeutic.

Generally, the formulations are prepared by contacting the Therapeutic uniformly and intimately with liquid carriers or finely divided solid carriers or both.

5 Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

10 The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten  
15 residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar  
20 alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The Therapeutic is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or  
25 stabilizers will result in the formation of polypeptide salts.

Any pharmaceutical used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutics generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a  
30 stopper pierceable by a hypodermic injection needle.

Therapeutics ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized



formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous Therapeutic solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized Therapeutic using bacteriostatic Water-for-Injection.

5           The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the Therapeutics of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use  
10       or sale for human administration. In addition, the Therapeutics may be employed in conjunction with other therapeutic compounds.

          The Therapeutics of the invention may be administered alone or in combination with adjuvants. Adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, alum, alum plus  
15       deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG, and MPL. In a specific embodiment, Therapeutics of the invention are administered in combination with alum. In another specific embodiment, Therapeutics of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to,  
20       Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the Therapeutics of the invention include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diphtheria, hepatitis A, hepatitis B, haemophilus influenzae B,  
25       whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic  
30       mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate



administration of one of the compounds or agents given first, followed by the second.

The Therapeutics of the invention may be administered alone or in combination with other therapeutic agents. Therapeutic agents that may be administered in combination with the Therapeutics of the invention, include but not  
5 limited to, other members of the TNF family, chemotherapeutic agents, antibiotics, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, cytokines and/or growth factors. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are  
10 administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

15 In one embodiment, the Therapeutics of the invention are administered in combination with members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the Therapeutics of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta),  
20 OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), endokine-alpha (International Publication No. WO 98/07880), TR6 (International Publication No. WO 98/30694), OPG, and neutrokin-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF),  
25 and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International  
30 Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.



In certain embodiments, Therapeutics of the invention are administered in combination with antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors. Nucleoside reverse transcriptase inhibitors that may be administered in combination

5 with the Therapeutics of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). Non-nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but

10 are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents,

15 nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with Therapeutics of the invention to treat AIDS and/or to prevent or treat HIV infection.

In other embodiments, Therapeutics of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that

20 may be administered in combination with the Therapeutics of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™,

25 FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, Therapeutics of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™,

30 DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat or prevent an opportunistic *Pneumocystis carinii* pneumonia infection. In



another specific embodiment, Therapeutics of the invention are used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat or prevent an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment,

5 Therapeutics of the invention are used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat or prevent an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat

10 or prevent an opportunistic cytomegalovirus infection. In another specific embodiment, Therapeutics of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to prophylactically treat or prevent an opportunistic fungal infection. In another specific embodiment, Therapeutics of the invention are used in any combination with

15 ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat or prevent an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, Therapeutics of the invention are used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat or prevent an opportunistic *Toxoplasma gondii* infection. In another specific embodiment,

20 Therapeutics of the invention are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat or prevent an opportunistic bacterial infection.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered

25 with the Therapeutics of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the Therapeutics of the invention include, but are not limited to, amoxicillin,

30 beta-lactamases, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin,



erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamthoxazole, and vancomycin.

Conventional nonspecific immunosuppressive agents, that may be administered in combination with the Therapeutics of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide methylprednisone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells.

In specific embodiments, Therapeutics of the invention are administered in combination with immunosuppressants. Immunosuppressants preparations that may be administered with the Therapeutics of the invention include, but are not limited to, ORTHOCLONE™ (OKT3), SANDIMMUNE™/NEORAL™/SANGDYA™ (cyclosporin), PROGRAFT™ (tacrolimus), CELLCEPT™ (mycophenolate), Azathioprine, glucocorticosteroids, and RAPAMUNE™ (sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

In an additional embodiment, Therapeutics of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the Therapeutics of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, and GAMIMUNE™. In a specific embodiment, Therapeutics of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

In an additional embodiment, the Therapeutics of the invention are administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the Therapeutics of the invention include, but are not limited to, glucocorticoids and the nonsteroidal anti-inflammatories, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-



acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

5 In another embodiment, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the Therapeutics of the invention include, but are not limited to, antibiotic derivatives (e.g., doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate, floxuridine, interferon alpha-2b, glutamic acid, plicamycin, mercaptopurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate); hormones (e.g., medroxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g., mephallen, chorambucil, mechlorethamine (nitrogen mustard) and thiotepa); steroids and combinations (e.g., bethamethasone sodium phosphate); and others (e.g., dicarbazine, asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, and etoposide).

In a specific embodiment, Therapeutics of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or any combination of the components of CHOP. In another embodiment, Therapeutics of the invention are administered in combination with Rituximab. In a further embodiment, Therapeutics of the invention are administered with Rituxmab and CHOP, or Rituxmab and any combination of the components of CHOP.

In an additional embodiment, the Therapeutics of the invention are administered in combination with cytokines. Cytokines that may be administered with the Therapeutics of the invention include, but are not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha. In another embodiment, Therapeutics of the invention may be administered



with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21.

In an additional embodiment, the Therapeutics of the invention are administered in combination with angiogenic proteins. Angiogenic proteins that may be administered with the Therapeutics of the invention include, but are not limited to, Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number EP-399816; Platelet Derived Growth Factor-A (PDGF-A), as disclosed in European Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as disclosed in European Patent Number EP-282317; Placental Growth Factor (PlGF), as disclosed in International Publication Number WO 92/06194; Placental Growth Factor-2 (PlGF-2), as disclosed in Hauser et al., Growth Factors, 4:259-268 (1993); Vascular Endothelial Growth Factor (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth Factor-2 (VEGF-2), as disclosed in International Publication Number WO 96/39515; Vascular Endothelial Growth Factor B (VEGF-3); Vascular Endothelial Growth Factor B-186 (VEGF-B186), as disclosed in International Publication Number WO 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/02543; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German Patent Number DE19639601. The above mentioned references are incorporated herein by reference herein.

In an additional embodiment, the Therapeutics of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the Therapeutics of the invention include, but are not limited to, LEUKINE™ (SARGRAMOSTIM™) and NEUPOGEN™ (FILGRASTIM™).

In an additional embodiment, the Therapeutics of the invention are administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be administered with the Therapeutics of the invention include, but



are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

In additional embodiments, the Therapeutics of the invention are administered in combination with other therapeutic or prophylactic regimens, such as, for example,  
5 radiation therapy.

**Example 24: Method of Treating Decreased Levels of the Polypeptide**

The present invention relates to a method for treating an individual in need of an increased level of a polypeptide of the invention in the body comprising  
10 administering to such an individual a composition comprising a therapeutically effective amount of an agonist of the invention (including polypeptides of the invention). Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of a secreted protein in an individual can be treated by administering the polypeptide of the present invention, preferably in the  
15 secreted form. Thus, the invention also provides a method of treatment of an individual in need of an increased level of the polypeptide comprising administering to such an individual a Therapeutic comprising an amount of the polypeptide to increase the activity level of the polypeptide in such an individual.

For example, a patient with decreased levels of a polypeptide receives a daily  
20 dose 0.1-100 ug/kg of the polypeptide for six consecutive days. Preferably, the polypeptide is in the secreted form. The exact details of the dosing scheme, based on administration and formulation, are provided in Example 23.

**Example 25: Method of Treating Increased Levels of the Polypeptide**

25 The present invention also relates to a method of treating an individual in need of a decreased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an antagonist of the invention (including polypeptides and antibodies of the invention).

30 In one example, antisense technology is used to inhibit production of a polypeptide of the present invention. This technology is one example of a method of



decreasing levels of a polypeptide, preferably a secreted form, due to a variety of etiologies, such as cancer. For example, a patient diagnosed with abnormally increased levels of a polypeptide is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation of the antisense polynucleotide is provided in Example 23.

#### **Example 26: Method of Treatment Using Gene Therapy-Ex Vivo**

One method of gene therapy transplants fibroblasts, which are capable of expressing a polypeptide, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37 degree C for approximately one week.

At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

The cDNA encoding a polypeptide of the present invention can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 1 using primers and having appropriate restriction sites and initiation/stop codons, if necessary. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is



maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector has the gene of interest properly inserted.

- 5           The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the gene (the
- 10          packaging cells are now referred to as producer cells).

- Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is
- 15          removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been
- 20          efficiently infected, the fibroblasts are analyzed to determine whether protein is produced.

          The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

25       **Example 27: Gene Therapy Using Endogenous Genes Corresponding To Polynucleotides of the Invention**

- Another method of gene therapy according to the present invention involves operably associating the endogenous polynucleotide sequence of the invention with a promoter via homologous recombination as described, for example, in U.S. Patent
- 30       NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., *Proc. Natl. Acad. Sci. USA*, 86:8932-8935



(1989); and Zijlstra et al., *Nature*, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of endogenous polynucleotide sequence, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of the polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter.

The amplified promoter and the amplified targeting sequences are digested with the appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The digested promoter and digested targeting sequences are added together in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel then purified by phenol extraction and ethanol precipitation.

In this Example, the polynucleotide constructs are administered as naked polynucleotides via electroporation. However, the polynucleotide constructs may also be administered with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous polynucleotide sequence. This results in the expression of polynucleotide corresponding to the polynucleotide in the cell. Expression may be detected by immunological staining, or any other method known in the art.



Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An aliquot of the cell suspension is removed for counting, and the remaining

5 cells are subjected to centrifugation. The supernatant is aspirated and the pellet is resuspended in 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM Na<sub>2</sub> HPO<sub>4</sub>, 6 mM dextrose). The cells are recentrifuged, the supernatant aspirated, and the cells resuspended in electroporation buffer containing 1 mg/ml acetylated bovine serum albumin. The final cell suspension contains

10 approximately 3X10<sup>6</sup> cells/ml. Electroporation should be performed immediately following resuspension.

Plasmid DNA is prepared according to standard techniques. For example, to construct a plasmid for targeting to the locus corresponding to the polynucleotide of the invention, plasmid pUC18 (MBI Fermentas, Amherst, NY) is digested with

15 HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3'end. Two non-coding sequences are amplified via PCR: one non-coding sequence (fragment 1) is amplified with a HindIII site at the 5' end and an Xba site at the 3'end; the other non-coding sequence (fragment 2) is amplified with a BamHI site at the 5'end and a HindIII site at the 3'end. The CMV promoter and the

20 fragments (1 and 2) are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; fragment 1 - XbaI; fragment 2 - BamHI) and ligated together. The resulting ligation product is digested with HindIII, and ligated with the HindIII-digested pUC18 plasmid.

Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap

25 (Bio-Rad). The final DNA concentration is generally at least 120 µg/ml. 0.5 ml of the cell suspension (containing approximately 1.5X10<sup>6</sup> cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960 µF and 250-300 V, respectively. As voltage increases, cell survival decreases, but

30 the percentage of surviving cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should be observed.



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### **Example 28: Method of Treatment Using Gene Therapy - In Vivo**

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precipitating agents and the like. However, the polynucleotides of the present invention may also be delivered in liposome formulations (such as those taught in Felgner P.L. et al. (1995) Ann. NY Acad. Sci. 772:126-139 and Abdallah B. et al. (1995) Biol. Cell 85(1):1-7) which can be prepared by methods well known to those skilled in the art.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg



body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence  
 5 can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or  
 10 mucous membranes of the nose. In addition, naked polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The dose response effects of injected polynucleotide in muscle *in vivo* is determined as follows. Suitable template DNA for production of mRNA coding for polypeptide of the present invention is prepared in accordance with a standard  
 15 recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on  
 20 the anterior thigh, and the quadriceps muscle is directly visualized. The template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

25 After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15  $\mu$ m cross-section of the individual quadriceps muscles is histochemically stained for protein expression. A time course for protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of DNA  
 30 in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be use to extrapolate proper



dosages and other treatment parameters in humans and other animals using naked DNA.

### Example 29: Transgenic Animals.

5           The polypeptides of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, *e.g.*, baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy  
10           protocol.

Any technique known in the art may be used to introduce the transgene (i.e., polynucleotides of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson et al., Appl. Microbiol. Biotechnol. 40:691-698 (1994); Carver et al., Biotechnology (NY) 11:1263-1270 (1993); Wright et al., Biotechnology (NY) 9:830-834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci., USA 82:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson et al., Cell 56:313-321 (1989)); electroporation of cells or embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, e.g., Ulmer et al., Science 259:1745 (1993); introducing nucleic acid constructs into embryonic pluripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano et al., Cell 57:717-723 (1989); etc. For a review of such techniques, see Gordon, "Transgenic Animals," Intl. Rev. Cytol. 115:171-229 (1989), which is incorporated by reference herein in its entirety.

Any technique known in the art may be used to produce transgenic clones containing polynucleotides of the invention, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campell et al., Nature 380:64-66 (1996); Wilmut et al., Nature 385:810-813 (1997)).



The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, *i.e.*, mosaic animals or chimeric. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, *e.g.*, head-to-head tandems or  
5 head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When  
10 it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the  
15 nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent  
20 to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA  
25 expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies  
30 specific for the transgene product.

Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding



strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest.

Transgenic animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying diseases, disorders, and/or conditions associated with aberrant expression, and in screening for compounds effective in ameliorating such diseases, disorders, and/or conditions.

### **Example 30: Knock-Out Animals.**

Endogenous gene expression can also be reduced by inactivating or "knocking out" the gene and/or its promoter using targeted homologous recombination. (*E.g.*, see Smithies et al., Nature 317:230-234 (1985); Thomas & Capecchi, Cell 51:503-512 (1987); Thompson et al., Cell 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express polypeptides of the invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (*e.g.*, see Thomas &



Capecchi 1987 and Thompson 1989, *supra*). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art.

5 In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (e.g., knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (i.e., animal, including human) or an MHC compatible donor and can include, but are not  
10 limited to fibroblasts, bone marrow cells, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention,  
15 e.g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve  
20 expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally.

Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For



example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

Transgenic and “knock-out” animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying diseases, disorders, and/or conditions associated with aberrant expression, and in screening for compounds effective in ameliorating such diseases, disorders, and/or conditions.

### 10 **Example 31: Production of an Antibody**

#### a) Hybridoma Technology

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing polypeptide(s) of the invention are administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of polypeptide(s) of the invention is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

Monoclonal antibodies specific for polypeptide(s) of the invention are prepared using hybridoma technology. (Kohler et al., Nature 256:495 (1975); Kohler et al., Eur. J. Immunol. 6:511 (1976); Kohler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981)). In general, an animal (preferably a mouse) is immunized with polypeptide(s) of the invention, or, more preferably, with a secreted polypeptide-expressing cell. Such polypeptide-expressing cells are cultured in any suitable tissue culture medium, preferably in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line



(SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide(s) of the invention.

Alternatively, additional antibodies capable of binding polypeptide(s) of the invention can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the polypeptide(s) of the invention protein-specific antibody can be blocked by polypeptide(s) of the invention. Such antibodies comprise anti-idiotypic antibodies to the polypeptide(s) of the invention protein-specific antibody and are used to immunize an animal to induce formation of further polypeptide(s) of the invention protein-specific antibodies.

For in vivo use of antibodies in humans, an antibody is "humanized". Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric and humanized antibodies are known in the art and are discussed herein. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

#### b) Isolation Of Antibody Fragments Directed

polypeptide(s) of the invention From A Library Of scFvs

Naturally occurring V-genes isolated from human PBLs are constructed into a library of antibody fragments which contain reactivities against polypeptide(s) of the



invention to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein by reference in its entirety).

Rescue of the Library. A library of scFvs is constructed from the RNA of human PBLs as described in PCT publication WO 92/01047. To rescue phage displaying antibody fragments, approximately 10<sup>9</sup> E. coli harboring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 µg/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to inoculate 50 ml of 2xTY-AMP-GLU, 2 x 10<sup>8</sup> TU of delta gene 3 helper (M13 delta gene III, see PCT publication WO 92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2xTY containing 100 µg/ml ampicillin and 50 ug/ml kanamycin and grown overnight. Phage are prepared as described in PCT publication WO 92/01047.

15 M13 delta gene III is prepared as follows: M13 delta gene III helper phage  
does not encode gene III protein, hence the phage(mid) displaying antibody  
fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III  
particles are made by growing the helper phage in cells harboring a pUC19 derivative  
supplying the wild type gene III protein during phage morphogenesis. The culture is  
20 incubated for 1 hour at 37° C without shaking and then for a further hour at 37°C with  
shaking. Cells are spun down (IEC-Centra 8,400 r.p.m. for 10 min), resuspended in  
300 ml 2xTY broth containing 100 µg ampicillin/ml and 25 µg kanamycin/ml (2xTY-  
AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and  
concentrated from the culture medium by two PEG-precipitations (Sambrook et al.,  
25 1990), resuspended in 2 ml PBS and passed through a 0.45 µm filter (Minisart NML;  
Sartorius) to give a final concentration of approximately 10<sup>13</sup> transducing units/ml  
(ampicillin-resistant clones).

Panning of the Library. Immuntubes (Nunc) are coated overnight in PBS with 4 ml of either 100  $\mu\text{g/ml}$  or 10  $\mu\text{g/ml}$  of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS. Approximately  $10^{13}$  TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to



stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The E. coli are then plated on TYE plates containing 1% glucose and 100 µg/ml ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

Characterization of Binders. Eluted phage from the 3rd and 4th rounds of selection are used to infect E. coli HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtitre plates coated with either 10 pg/ml of the polypeptide of the present invention in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see, e.g., PCT publication WO 92/01047) and then by sequencing. These ELISA positive clones may also be further characterized by techniques known in the art, such as, for example, epitope mapping, binding affinity, receptor signal transduction, ability to block or competitively inhibit antibody/antigen binding, and competitive agonistic or antagonistic activity.

### **Example 32: Assays Detecting Stimulation or Inhibition of B cell Proliferation and Differentiation**

Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL-5, IL-6, IL-7, IL10, IL-13, IL-14 and IL-15. Interestingly, these signals are by themselves weak effectors but can,



in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations.

One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the detection of the differentiation, proliferation, or inhibition of B-cell populations and their precursors.

In Vitro Assay- Purified polypeptides of the invention, or truncated forms thereof, is assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of the polypeptides of the invention on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220).

Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added  $10^5$  B-cells suspended in culture medium (RPMI 1640 containing 10% FBS,  $5 \times 10^{-5}$ M 2ME, 100U/ml penicillin, 10ug/ml streptomycin, and  $10^{-5}$  dilution of SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated by a 20h pulse (1uCi/well) with 3H-thymidine (6.7 Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively.

In Vivo Assay- BALB/c mice are injected (i.p.) twice per day with buffer only, or 2 mg/Kg of a polypeptide of the invention, or truncated forms thereof. Mice



receive this treatment for 4 consecutive days, at which time they are sacrificed and various tissues and serum collected for analyses. Comparison of H&E sections from normal spleens and spleens treated with polypeptides of the invention identify the results of the activity of the polypeptides on spleen cells, such as the diffusion of peri-arterial lymphatic sheaths, and/or significant increases in the nucleated cellularity of the red pulp regions, which may indicate the activation of the differentiation and proliferation of B-cell populations. Immunohistochemical studies using a B cell marker, anti-CD45R(B220), are used to determine whether any physiological changes to splenic cells, such as splenic disorganization, are due to increased B-cell representation within loosely defined B-cell zones that infiltrate established T-cell regions.

Flow cytometric analyses of the spleens from mice treated with polypeptide is used to indicate whether the polypeptide specifically increases the proportion of ThB+, CD45R(B220)dull B cells over that which is observed in control mice.

Likewise, a predicted consequence of increased mature B-cell representation in vivo is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are compared between buffer and polypeptide-treated mice.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

### **Example 33: T Cell Proliferation Assay**

#### **Proliferation assay for Resting PBLs.**

A CD3-induced proliferation assay is performed on PBMCs and is measured by the uptake of <sup>3</sup>H-thymidine. The assay is performed as follows. Ninety-six well plates are coated with 100 microliters per well of mAb to CD3 (HIT3a, Pharmingen) or isotype-matched control mAb (B33.1) overnight at 4 °C (1 microgram/ml in .05M bicarbonate buffer, pH 9.5), then washed three times with PBS. PBMC are isolated by F/H gradient centrifugation from human peripheral blood and added to quadruplicate wells (5 x 10<sup>4</sup>/well) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence of varying concentrations of TNF Delta and/or TNF Epsilon protein (total volume 200 microliters). Relevant protein buffer and medium alone are controls.



After 48 hr. culture at 37 °C, plates are spun for 2 min. at 1000 rpm and 100 microliters of supernatant is removed and stored -20 °C for measurement of IL-2 (or other cytokines) if effect on proliferation is observed. Wells are supplemented with 100 microliters of medium containing 0.5 microcuries of <sup>3</sup>H-thymidine and cultured at 37 °C for 18-24 hr. Wells are harvested and

5 incorporation of <sup>3</sup>H-thymidine used as a measure of proliferation. Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is also used as a control which enhances proliferation. Control antibody which does not induce proliferation of T cells is used as the negative controls for the effects of TNF Delta and/or TNF Epsilon proteins.

Alternatively, a proliferation assay on resting PBL (peripheral blood

10 lymphocytes) is measured by the up-take of <sup>3</sup>H-thymidine. The assay is performed as follows. PBMC are isolated by Ficoll (LSM, ICN Biotechnologies, Aurora, Ohio) gradient centrifugation from human peripheral blood, and are cultured overnight in 10% (Fetal Calf Serum, Biofluids, Rockville, MD)/RPMI (Gibco BRL, Gaithersburg, MD). This overnight incubation period allows the adherent cells to attach to the

15 plastic, which results in a lower background in the assay as there are fewer cells that can act as antigen presenting cells or that might be producing growth factors. The following day the non-adherent cells are collected, washed and used in the proliferation assay. The assay is performed in a 96 well plate using 2 x 10<sup>4</sup> cells/well in a final volume of 200 microliters. The supernatants (e.g., CHO or 293T

20 supernatants) expressing the protein of interest are tested at a 30% final dilution, therefore 60ul are added to 140ul of 10% FCS/RPMI containing the cells. Control supernatants are used at the same final dilution and express the following proteins: vector (negative control), IL-2 (\*), IFN $\gamma$ , TNF $\alpha$ , IL-10 and TR2. In addition to the control supernatants, recombinant human IL-2 (R & D Systems, Minneapolis, MN)

25 at a final concentration of 100ng/ml is also used. After 24 hours of culture, each well is pulsed with 1uCi of <sup>3</sup>H-thymidine (Nen, Boston, MA). Cells are then harvested 20 hours following pulsing and incorporation of <sup>3</sup>H-thymidine is used as a measure of proliferation. Results are expressed as an average of triplicate samples plus or minus standard error.

30 (\*) The amount of the control cytokines IL-2, IFN $\gamma$ , TNF $\alpha$  and IL-10 produced in each transfection varies between 300pg to 5ng/ml.



### Costimulation assay.

A costimulation assay on resting PBL (peripheral blood lymphocytes) is performed in the presence of immobilized antibodies to CD3 and CD28. The use of antibodies specific for the invariant regions of CD3 mimic the induction of T cell activation that would occur through stimulation of the T cell receptor by an antigen. Cross-linking of the TCR (first signal) in the absence of a costimulatory signal (second signal) causes very low induction of proliferation and will eventually result in a state of "anergy", which is characterized by the absence of growth and inability to produce cytokines. The addition of a costimulatory signal such as an antibody to CD28, which mimics the action of the costimulatory molecule. B7-1 expressed on activated APCs, results in enhancement of T cell responses including cell survival and production of IL-2. Therefore this type of assay allows to detect both positive and negative effects caused by addition of supernatants expressing the proteins of interest on T cell proliferation.

The assay is performed as follows. Ninety-six well plates are coated with 100ng/ml anti-CD3 and 5ug/ml anti-CD28 (Pharmingen, San Diego, CA) in a final volume of 100ul and incubated overnight at 4C. Plates are washed twice with PBS before use. PBMC are isolated by Ficoll (LSM, ICN Biotechnologies, Aurora, Ohio) gradient centrifugation from human peripheral blood, and are cultured overnight in 10% FCS(Fetal Calf Serum, Biofluids, Rockville, MD)/RPMI (Gibco BRL, Gaithersburg, MD). This overnight incubation period allows the adherent cells to attach to the plastic, which results in a lower background in the assay as there are fewer cells that can act as antigen presenting cells or that might be producing growth factors. The following day the non adherent cells are collected, washed and used in the proliferation assay. The assay is performed in a 96 well plate using  $2 \times 10^4$  cells/well in a final volume of 200ul. The supernatants (e.g., CHO or 293T supernatants) expressing the protein of interest are tested at a 30% final dilution, therefore 60ul are added to 140ul of 10% FCS/RPMI containing the cells. Control supernatants are used at the same final dilution and express the following proteins: vector only (negative control), IL-2, IFN $\gamma$ , TNF $\alpha$ , IL-10 and TR2. In addition to the control supernatants recombinant human IL-2 (R & D Systems, Minneapolis, MN) at a final concentration of 10ng/ml is also used. After 24 hours of culture, each well is



pulsed with 1uCi of  $^3\text{H}$ -thymidine (Nen, Boston, MA). Cells are then harvested 20 hours following pulsing and incorporation of  $^3\text{H}$ -thymidine is used as a measure of proliferation. Results are expressed as an average of triplicate samples plus or minus standard error.

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#### **Proliferation assay for preactivated-resting T cells.**

A proliferation assay on preactivated-resting T cells is performed on cells that are previously activated with the lectin phytohemagglutinin (PHA). Lectins are polymeric plant proteins that can bind to residues on T cell surface glycoproteins including the TCR and act as polyclonal activators. PBLs treated with PHA and then cultured in the presence of low doses of IL-2 resemble effector T cells. These cells are generally more sensitive to further activation induced by growth factors such as IL-2. This is due to the expression of high affinity IL-2 receptors that allows this population to respond to amounts of IL-2 that are 100 fold lower than what would have an effect on a naïve T cell. Therefore the use of this type of cells might enable to detect the effect of very low doses of an unknown growth factor, that would not be sufficient to induce proliferation on resting (naïve ) T cells.

The assay is performed as follows. PBMC are isolated by F/H gradient centrifugation from human peripheral blood, and are cultured in 10% FCS (Fetal Calf Serum, Biofluids, Rockville, MD)/RPMI (Gibco BRL, Gaithersburg, MD) in the presence of 2ug/ml PHA (Sigma, Saint Louis, MO) for three days. The cells are then washed in PBS and cultured in 10% FCS/RPMI in the presence of 5ng/ml of human recombinant IL-2 (R & D Systems, Minneapolis, MN) for 3 days. The cells are washed and rested in starvation medium (1%FCS/RPMI) for 16 hours prior to the beginning of the proliferation assay. An aliquot of the cells is analyzed by FACS to determine the percentage of T cells (CD3 positive cells) present; this usually ranges between 93-97% depending on the donor. The assay is performed in a 96 well plate using  $2 \times 10^4$  cells/well in a final volume of 200ul. The supernatants (e.g., CHO or 293T supernatants) expressing the protein of interest are tested at a 30% final dilution, therefore 60ul are added to 140ul of in 10% FCS/RPMI containing the cells. Control supernatants are used at the same final dilution and express the following proteins: vector (negative control), IL-2, IFN $\gamma$ , TNF $\alpha$ , IL-10 and TR2. In addition to the



control supernatants recombinant human IL-2 at a final concentration of 10ng/ml is also used. After 24 hours of culture, each well is pulsed with 1uCi of  $^3\text{H}$ -thymidine(Nen, Boston, MA). Cells are then harvested 20 hours following pulsing and incorporation of  $^3\text{H}$ -thymidine is used as a measure of proliferation. Results are expressed as an average of triplicate samples plus or minus standard error.

The studies described in this example test activity of polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

**Example 34: Effect of Polypeptides of the Invention on the Expression of MHC Class II, Costimulatory and Adhesion Molecules and Cell Differentiation of Monocytes and Monocyte-Derived Human Dendritic Cells**

Dendritic cells are generated by the expansion of proliferating precursors found in the peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for 7-10 days with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). These dendritic cells have the characteristic phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and MHC class II antigens). Treatment with activating factors, such as TNF- $\alpha$ , causes a rapid change in surface phenotype (increased expression of MHC class I and II, costimulatory and adhesion molecules, downregulation of FC $\gamma$ RII, upregulation of CD83). These changes correlate with increased antigen-presenting capacity and with functional maturation of the dendritic cells.

FACS analysis of surface antigens is performed as follows. Cells are treated 1-3 days with increasing concentrations of polypeptides of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

Effect on the production of cytokines. Cytokines generated by dendritic cells, in particular IL-12, are important in the initiation of T-cell dependent immune



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gradient (Sigma). Monocytes are isolated from PBMC by counterflow centrifugal elutriation.

Monocyte Survival Assay. Human peripheral blood monocytes progressively lose viability when cultured in absence of serum or other stimuli. Their death results from internally regulated process (apoptosis). Addition to the culture of activating factors, such as TNF-alpha dramatically improves cell survival and prevents DNA fragmentation. Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are cultured for 48 hours in polypropylene tubes in serum-free medium (positive control), in the presence of 100 ng/ml TNF-alpha (negative control), and in the presence of varying concentrations of the compound to be tested. Cells are suspended at a concentration of  $2 \times 10^6$ /ml in PBS containing PI at a final concentration of 5  $\mu$ g/ml, and then incubated at room temperature for 5 minutes before FACScan analysis. PI uptake has been demonstrated to correlate with DNA fragmentation in this experimental paradigm.

Effect on cytokine release. An important function of monocytes/macrophages is their regulatory activity on other cellular populations of the immune system through the release of cytokines after stimulation. An ELISA to measure cytokine release is performed as follows. Human monocytes are incubated at a density of  $5 \times 10^5$  cells/ml with increasing concentrations of the a polypeptide of the invention and under the same conditions, but in the absence of the polypeptide. For IL-12 production, the cells are primed overnight with IFN (100 U/ml) in presence of a polypeptide of the invention. LPS (10 ng/ml) is then added. Conditioned media are collected after 24h and kept frozen until use. Measurement of TNF-alpha, IL-10, MCP-1 and IL-8 is then performed using a commercially available ELISA kit (e.g, R & D Systems (Minneapolis, MN)) and applying the standard protocols provided with the kit.

Oxidative burst. Purified monocytes are plated in 96-w plate at  $2 \cdot 10^5$  cell/well. Increasing concentrations of polypeptides of the invention are added to the wells in a total volume of 0.2 ml culture medium (RPMI 1640 + 10% FCS, glutamine and antibiotics). After 3 days incubation, the plates are centrifuged and the medium is



removed from the wells. To the macrophage monolayers, 0.2 ml per well of phenol red solution (140 mM NaCl, 10 mM potassium phosphate buffer pH 7.0, 5.5 mM dextrose, 0.56 mM phenol red and 19 U/ml of HRPO) is added, together with the stimulant (200 nM PMA). The plates are incubated at 37°C for 2 hours and the reaction is stopped by adding 20 µl 1N NaOH per well. The absorbance is read at 610 nm. To calculate the amount of H<sub>2</sub>O<sub>2</sub> produced by the macrophages, a standard curve of a H<sub>2</sub>O<sub>2</sub> solution of known molarity is performed for each experiment.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polypeptides, polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

### **Example 35: Biological Effects of Polypeptides of the Invention**

#### **Astrocyte and Neuronal Assays**

Recombinant polypeptides of the invention, expressed in *Escherichia coli* and purified as described above, can be tested for activity in promoting the survival, neurite outgrowth, or phenotypic differentiation of cortical neuronal cells and for inducing the proliferation of glial fibrillary acidic protein immunopositive cells, astrocytes. The selection of cortical cells for the bioassay is based on the prevalent expression of FGF-1 and FGF-2 in cortical structures and on the previously reported enhancement of cortical neuronal survival resulting from FGF-2 treatment. A thymidine incorporation assay, for example, can be used to elucidate a polypeptide of the invention's activity on these cells.

Moreover, previous reports describing the biological effects of FGF-2 (basic FGF) on cortical or hippocampal neurons *in vitro* have demonstrated increases in both neuron survival and neurite outgrowth (Walicke et al., "Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension." *Proc. Natl. Acad. Sci. USA* 83:3012-3016. (1986), assay herein incorporated by reference in its entirety). However, reports from experiments done on PC-12 cells suggest that these two responses are not necessarily synonymous and may depend on not only which FGF is being tested but also on which receptor(s) are expressed on the target cells. Using the primary cortical neuronal culture paradigm, the ability of a polypeptide of the invention to



induce neurite outgrowth can be compared to the response achieved with FGF-2 using, for example, a thymidine incorporation assay.

#### Fibroblast and endothelial cell assays

Human lung fibroblasts are obtained from Clonetics (San Diego, CA) and maintained in growth media from Clonetics. Dermal microvascular endothelial cells are obtained from Cell Applications (San Diego, CA). For proliferation assays, the human lung fibroblasts and dermal microvascular endothelial cells can be cultured at 5,000 cells/well in a 96-well plate for one day in growth medium. The cells are then incubated for one day in 0.1% BSA basal medium. After replacing the medium with fresh 0.1% BSA medium, the cells are incubated with the test proteins for 3 days. Alamar Blue (Alamar Biosciences, Sacramento, CA) is added to each well to a final concentration of 10%. The cells are incubated for 4 hr. Cell viability is measured by reading in a CytoFluor fluorescence reader. For the PGE<sub>2</sub> assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or polypeptides of the invention with or without IL-1 $\alpha$  for 24 hours. The supernatants are collected and assayed for PGE<sub>2</sub> by EIA kit (Cayman, Ann Arbor, MI). For the IL-6 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or with or without polypeptides of the invention IL-1 $\alpha$  for 24 hours. The supernatants are collected and assayed for IL-6 by ELISA kit (Endogen, Cambridge, MA).

Human lung fibroblasts are cultured with FGF-2 or polypeptides of the invention for 3 days in basal medium before the addition of Alamar Blue to assess effects on growth of the fibroblasts. FGF-2 should show a stimulation at 10 - 2500 ng/ml which can be used to compare stimulation with polypeptides of the invention.

#### Parkinson Models.

The loss of motor function in Parkinson's disease is attributed to a deficiency of striatal dopamine resulting from the degeneration of the nigrostriatal dopaminergic



projection neurons. An animal model for Parkinson's that has been extensively characterized involves the systemic administration of 1-methyl-4 phenyl 1,2,3,6-tetrahydropyridine (MPTP). In the CNS, MPTP is taken-up by astrocytes and catabolized by monoamine oxidase B to 1-methyl-4-phenyl pyridine (MPP<sup>+</sup>) and released. Subsequently, MPP<sup>+</sup> is actively accumulated in dopaminergic neurons by the high-affinity reuptake transporter for dopamine. MPP<sup>+</sup> is then concentrated in mitochondria by the electrochemical gradient and selectively inhibits nicotinamide adenine disphosphate: ubiquinone oxidoreductionase (complex I), thereby interfering with electron transport and eventually generating oxygen radicals.

It has been demonstrated in tissue culture paradigms that FGF-2 (basic FGF) has trophic activity towards nigral dopaminergic neurons (Ferrari et al., Dev. Biol. 1989). Recently, Dr. Unsicker's group has demonstrated that administering FGF-2 in gel foam implants in the striatum results in the near complete protection of nigral dopaminergic neurons from the toxicity associated with MPTP exposure (Otto and Unsicker, J. Neuroscience, 1990).

Based on the data with FGF-2, polypeptides of the invention can be evaluated to determine whether it has an action similar to that of FGF-2 in enhancing dopaminergic neuronal survival *in vitro* and it can also be tested *in vivo* for protection of dopaminergic neurons in the striatum from the damage associated with MPTP treatment. The potential effect of a polypeptide of the invention is first examined *in vitro* in a dopaminergic neuronal cell culture paradigm. The cultures are prepared by dissecting the midbrain floor plate from gestation day 14 Wistar rat embryos. The tissue is dissociated with trypsin and seeded at a density of 200,000 cells/cm<sup>2</sup> on polyorthinine-laminin coated glass coverslips. The cells are maintained in Dulbecco's Modified Eagle's medium and F12 medium containing hormonal supplements (N1). The cultures are fixed with paraformaldehyde after 8 days *in vitro* and are processed for tyrosine hydroxylase, a specific marker for dopaminergic neurons, immunohistochemical staining. Dissociated cell cultures are prepared from embryonic rats. The culture medium is changed every third day and the factors are also added at that time.

Since the dopaminergic neurons are isolated from animals at gestation day 14, a developmental time which is past the stage when the dopaminergic precursor cells are proliferating, an increase in the number of tyrosine hydroxylase immunopositive neurons



would represent an increase in the number of dopaminergic neurons surviving *in vitro*. Therefore, if a polypeptide of the invention acts to prolong the survival of dopaminergic neurons, it would suggest that the polypeptide may be involved in Parkinson's Disease.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 36: The Effect of Polypeptides of the Invention on the Growth of Vascular Endothelial Cells**

On day 1, human umbilical vein endothelial cells (HUVEC) are seeded at  $2.5 \times 10^4$  cells/35 mm dish density in M199 medium containing 4% fetal bovine serum (FBS), 16 units/ml heparin, and 50 units/ml endothelial cell growth supplements (ECGS, Biotechnology, Inc.). On day 2, the medium is replaced with M199 containing 10% FBS, 8 units/ml heparin. A polypeptide having the amino acid sequence of SEQ ID NO:Y, and positive controls, such as VEGF and basic FGF (bFGF) are added, at varying concentrations. On days 4 and 6, the medium is replaced. On day 8, cell number is determined with a Coulter Counter.

An increase in the number of HUVEC cells indicates that the polypeptide of the invention may proliferate vascular endothelial cells.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 37: Stimulatory Effect of Polypeptides of the Invention on the Proliferation of Vascular Endothelial Cells**

For evaluation of mitogenic activity of growth factors, the colorimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)2H-tetrazolium) assay with the electron coupling reagent PMS (phenazine methosulfate) was



performed (CellTiter 96 AQ, Promega). Cells are seeded in a 96-well plate (5,000 cells/well) in 0.1 mL serum-supplemented medium and are allowed to attach overnight. After serum-starvation for 12 hours in 0.5% FBS, conditions (bFGF, VEGF<sub>165</sub> or a polypeptide of the invention in 0.5% FBS) with or without Heparin (8 U/ml) are added to wells for 48 hours. 20 mg of MTS/PMS mixture (1:0.05) are added per well and allowed to incubate for 1 hour at 37°C before measuring the absorbance at 490 nm in an ELISA plate reader. Background absorbance from control wells (some media, no cells) is subtracted, and seven wells are performed in parallel for each condition. See, Leak *et al. In Vitro Cell. Dev. Biol.* 30A:512-518 (1994).

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 38: Inhibition of PDGF-induced Vascular Smooth Muscle Cell Proliferation Stimulatory Effect**

HAoSMC proliferation can be measured, for example, by BrdUrd incorporation. Briefly, subconfluent, quiescent cells grown on the 4-chamber slides are transfected with CRP or FITC-labeled AT2-3LP. Then, the cells are pulsed with 10% calf serum and 6 mg/ml BrdUrd. After 24 h, immunocytochemistry is performed by using BrdUrd Staining Kit (Zymed Laboratories). In brief, the cells are incubated with the biotinylated mouse anti-BrdUrd antibody at 4 degrees C for 2 h after being exposed to denaturing solution and then incubated with the streptavidin-peroxidase and diaminobenzidine. After counterstaining with hematoxylin, the cells are mounted for microscopic examination, and the BrdUrd-positive cells are counted. The BrdUrd index is calculated as a percent of the BrdUrd-positive cells to the total cell number. In addition, the simultaneous detection of the BrdUrd staining (nucleus) and the FITC uptake (cytoplasm) is performed for individual cells by the concomitant use of bright field illumination and dark field-UV fluorescent illumination. See, Hayashida et al., J. Biol. Chem. 6:271(36):21985-21992 (1996).



The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

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### **Example 39: Stimulation of Endothelial Migration**

This example will be used to explore the possibility that a polypeptide of the invention may stimulate lymphatic endothelial cell migration.

10        Endothelial cell migration assays are performed using a 48 well microchemotaxis chamber (Neuroprobe Inc., Cabin John, MD; Falk, W., et al., J. Immunological Methods 1980;33:239-247). Polyvinylpyrrolidone-free polycarbonate filters with a pore size of 8 um (Nucleopore Corp. Cambridge, MA) are coated with 0.1% gelatin for at least 6 hours at room temperature and dried under sterile air. Test substances are diluted to appropriate  
15        concentrations in M199 supplemented with 0.25% bovine serum albumin (BSA), and 25 ul of the final dilution is placed in the lower chamber of the modified Boyden apparatus. Subconfluent, early passage (2-6) HUVEC or BMEC cultures are washed and trypsinized for the minimum time required to achieve cell detachment. After placing the filter between lower and upper chamber,  $2.5 \times 10^5$  cells suspended in 50 ul M199 containing 1%  
20        FBS are seeded in the upper compartment. The apparatus is then incubated for 5 hours at 37°C in a humidified chamber with 5% CO<sub>2</sub> to allow cell migration. After the incubation period, the filter is removed and the upper side of the filter with the non-migrated cells is scraped with a rubber policeman. The filters are fixed with methanol and stained with a Giemsa solution (Diff-Quick, Baxter, McGraw Park, IL). Migration is quantified by  
25        counting cells of three random high-power fields (40x) in each well, and all groups are performed in quadruplicate.

30        The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

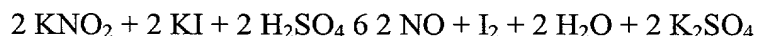


### Example 40: Stimulation of Nitric Oxide Production by Endothelial Cells

Nitric oxide released by the vascular endothelium is believed to be a mediator of vascular endothelium relaxation. Thus, activity of a polypeptide of the invention can be assayed by determining nitric oxide production by endothelial cells in response to the polypeptide.

Nitric oxide is measured in 96-well plates of confluent microvascular endothelial cells after 24 hours starvation and a subsequent 4 hr exposure to various levels of a positive control (such as VEGF-1) and the polypeptide of the invention. Nitric oxide in the medium is determined by use of the Griess reagent to measure total nitrite after reduction of nitric oxide-derived nitrate by nitrate reductase. The effect of the polypeptide of the invention on nitric oxide release is examined on HUVEC.

Briefly, NO release from cultured HUVEC monolayer is measured with a NO-specific polarographic electrode connected to a NO meter (Iso-NO, World Precision Instruments Inc.) (1049). Calibration of the NO elements is performed according to the following equation:



The standard calibration curve is obtained by adding graded concentrations of  $\text{KNO}_2$  (0, 5, 10, 25, 50, 100, 250, and 500 nmol/L) into the calibration solution containing KI and  $\text{H}_2\text{SO}_4$ . The specificity of the Iso-NO electrode to NO is previously determined by measurement of NO from authentic NO gas (1050). The culture medium is removed and HUVECs are washed twice with Dulbecco's phosphate buffered saline. The cells are then bathed in 5 ml of filtered Krebs-Henseleit solution in 6-well plates, and the cell plates are kept on a slide warmer (Lab Line Instruments Inc.) To maintain the temperature at  $37^\circ\text{C}$ . The NO sensor probe is inserted vertically into the wells, keeping the tip of the electrode 2 mm under the surface of the solution, before addition of the different conditions. S-nitroso acetyl penicillamin (SNAP) is used as a positive control. The amount of released NO is expressed as picomoles per  $1 \times 10^6$  endothelial cells. All values reported are means of four to six measurements in each group (number of cell culture wells). See, Leak *et al. Biochem. and Biophys. Res. Comm.* 217:96-105 (1995).

The studies described in this example tested activity of polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to



test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 41: Effect of Polypeptides of the Invention on Cord Formation in**

##### **Angiogenesis**

Another step in angiogenesis is cord formation, marked by differentiation of endothelial cells. This bioassay measures the ability of microvascular endothelial cells to form capillary-like structures (hollow structures) when cultured *in vitro*.

CADMEC (microvascular endothelial cells) are purchased from Cell Applications, Inc. as proliferating (passage 2) cells and are cultured in Cell Applications' CADMEC Growth Medium and used at passage 5. For the *in vitro* angiogenesis assay, the wells of a 48-well cell culture plate are coated with Cell Applications' Attachment Factor Medium (200 µl/well) for 30 min. at 37°C. CADMEC are seeded onto the coated wells at 7,500 cells/well and cultured overnight in Growth Medium. The Growth Medium is then replaced with 300 µg Cell Applications' Chord Formation Medium containing control buffer or a polypeptide of the invention (0.1 to 100 ng/ml) and the cells are cultured for an additional 48 hr. The numbers and lengths of the capillary-like chords are quantitated through use of the Boeckeler VIA-170 video image analyzer. All assays are done in triplicate.

Commercial (R&D) VEGF (50 ng/ml) is used as a positive control. b-esteradiol (1 ng/ml) is used as a negative control. The appropriate buffer (without protein) is also utilized as a control.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 42: Angiogenic Effect on Chick Chorioallantoic Membrane**

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Chick chorioallantoic membrane (CAM) is a well-established system to examine angiogenesis. Blood vessel formation on CAM is easily visible and quantifiable. The



ability of polypeptides of the invention to stimulate angiogenesis in CAM can be examined.

Fertilized eggs of the White Leghorn chick (*Gallus gallus*) and the Japanese quail (*Coturnix coturnix*) are incubated at 37.8°C and 80% humidity. Differentiated CAM of 16-day-old chick and 13-day-old quail embryos is studied with the following methods.

On Day 4 of development, a window is made into the egg shell of chick eggs. The embryos are checked for normal development and the eggs sealed with cellotape. They are further incubated until Day 13. Thermanox coverslips (Nunc, Naperville, IL) are cut into disks of about 5 mm in diameter. Sterile and salt-free growth factors are dissolved in distilled water and about 3.3 mg/ 5 ml are pipetted on the disks. After air-drying, the inverted disks are applied on CAM. After 3 days, the specimens are fixed in 3% glutaraldehyde and 2% formaldehyde and rinsed in 0.12 M sodium cacodylate buffer. They are photographed with a stereo microscope [Wild M8] and embedded for semi- and ultrathin sectioning as described above. Controls are performed with carrier disks alone.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 43: Angiogenesis Assay Using a Matrigel Implant in Mouse**

*In vivo* angiogenesis assay of a polypeptide of the invention measures the ability of an existing capillary network to form new vessels in an implanted capsule of murine extracellular matrix material (Matrigel). The protein is mixed with the liquid Matrigel at 4 degree C and the mixture is then injected subcutaneously in mice where it solidifies. After 7 days, the solid “plug” of Matrigel is removed and examined for the presence of new blood vessels. Matrigel is purchased from Becton Dickinson Labware/Collaborative Biomedical Products.

When thawed at 4 degree C the Matrigel material is a liquid. The Matrigel is mixed with a polypeptide of the invention at 150 ng/ml at 4 degrees C and drawn into cold 3 ml syringes. Female C57Bl/6 mice approximately 8 weeks old are injected with the



mixture of Matrigel and experimental protein at 2 sites at the midventral aspect of the abdomen (0.5 ml/site). After 7 days, the mice are sacrificed by cervical dislocation, the Matrigel plugs are removed and cleaned (i.e., all clinging membranes and fibrous tissue is removed). Replicate whole plugs are fixed in neutral buffered 10% formaldehyde, embedded in paraffin and used to produce sections for histological examination after staining with Masson's Trichrome. Cross sections from 3 different regions of each plug are processed. Selected sections are stained for the presence of vWF. The positive control for this assay is bovine basic FGF (150 ng/ml). Matrigel alone is used to determine basal levels of angiogenesis.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 44: Rescue of Ischemia in Rabbit Lower Limb Model**

To study the in vivo effects of polynucleotides and polypeptides of the invention on ischemia, a rabbit hindlimb ischemia model is created by surgical removal of one femoral arteries as described previously (Takeshita *et al.*, *Am J. Pathol* 147:1649-1660 (1995)). The excision of the femoral artery results in retrograde propagation of thrombus and occlusion of the external iliac artery. Consequently, blood flow to the ischemic limb is dependent upon collateral vessels originating from the internal iliac artery (Takeshita *et al.* *Am J. Pathol* 147:1649-1660 (1995)). An interval of 10 days is allowed for post-operative recovery of rabbits and development of endogenous collateral vessels. At 10 day post-operatively (day 0), after performing a baseline angiogram, the internal iliac artery of the ischemic limb is transfected with 500 mg naked expression plasmid containing a polynucleotide of the invention by arterial gene transfer technology using a hydrogel-coated balloon catheter as described (Riessen *et al.* *Hum Gene Ther.* 4:749-758 (1993); Leclerc *et al.* *J. Clin. Invest.* 90: 936-944 (1992)). When a polypeptide of the invention is used in the treatment, a single bolus of 500 mg polypeptide of the invention or control is delivered into the internal iliac artery of the ischemic limb over a period of 1



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exemplified studies to test the agonists, and/or antagonists of the invention.

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VIII immunohistochemistry or endothelial alkaline phosphatase reaction. Expression of



polypeptides of the invention, during the skin ischemia, is studied using in situ hybridization.

The study in this model is divided into three parts as follows:

- a) Ischemic skin
- b) Ischemic skin wounds
- c) Normal wounds

The experimental protocol includes:

- a) Raising a 3x4 cm, single pedicle full-thickness random skin flap (myocutaneous flap over the lower back of the animal).
- b) An excisional wounding (4-6 mm in diameter) in the ischemic skin (skin-flap).
- c) Topical treatment with a polypeptide of the invention of the excisional wounds (day 0, 1, 2, 3, 4 post-wounding) at the following various dosage ranges: 1mg to 100 mg.
- d) Harvesting the wound tissues at day 3, 5, 7, 10, 14 and 21 post-wounding for histological, immunohistochemical, and in situ studies.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 47: Peripheral Arterial Disease Model**

Angiogenic therapy using a polypeptide of the invention is a novel therapeutic strategy to obtain restoration of blood flow around the ischemia in case of peripheral arterial diseases. The experimental protocol includes:

- a) One side of the femoral artery is ligated to create ischemic muscle of the hindlimb, the other side of hindlimb serves as a control.
- b) a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-3 weeks.
- c) The ischemic muscle tissue is collected after ligation of the femoral



artery at 1, 2, and 3 weeks for the analysis of expression of a polypeptide of the invention and histology. Biopsy is also performed on the other side of normal muscle of the contralateral hindlimb.

5 The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### 10 **Example 48: Ischemic Myocardial Disease Model**

A polypeptide of the invention is evaluated as a potent mitogen capable of stimulating the development of collateral vessels, and restructuring new vessels after coronary artery occlusion. Alteration of expression of the polypeptide is investigated in situ. The experimental protocol includes:

15 a) The heart is exposed through a left-side thoracotomy in the rat. Immediately, the left coronary artery is occluded with a thin suture (6-0) and the thorax is closed.

b) a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-4 weeks.

20 c) Thirty days after the surgery, the heart is removed and cross-sectioned for morphometric and in situ analyzes.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the  
25 invention.

#### **Example 49: Rat Corneal Wound Healing Model**

30 This animal model shows the effect of a polypeptide of the invention on neovascularization. The experimental protocol includes:

a) Making a 1-1.5 mm long incision from the center of cornea into the stromal layer.



b) Inserting a spatula below the lip of the incision facing the outer corner of the eye.

c) Making a pocket (its base is 1-1.5 mm from the edge of the eye).

d) Positioning a pellet, containing 50ng- 5ug of a polypeptide of the invention, within the pocket.

e) Treatment with a polypeptide of the invention can also be applied topically to the corneal wounds in a dosage range of 20mg - 500mg (daily treatment for five days).

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 50: Diabetic Mouse and Glucocorticoid-Impaired Wound Healing Models**

##### ***A. Diabetic db+/db+ Mouse Model.***

To demonstrate that a polypeptide of the invention accelerates the healing process, the genetically diabetic mouse model of wound healing is used. The full thickness wound healing model in the db+/db+ mouse is a well characterized, clinically relevant and reproducible model of impaired wound healing. Healing of the diabetic wound is dependent on formation of granulation tissue and re-epithelialization rather than contraction (Gartner, M.H. *et al.*, *J. Surg. Res.* 52:389 (1992); Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)).

The diabetic animals have many of the characteristic features observed in Type II diabetes mellitus. Homozygous (db+/db+) mice are obese in comparison to their normal heterozygous (db+/+m) littermates. Mutant diabetic (db+/db+) mice have a single autosomal recessive mutation on chromosome 4 (db+) (Coleman *et al.* *Proc. Natl. Acad. Sci. USA* 77:283-293 (1982)). Animals show polyphagia, polydipsia and polyuria. Mutant diabetic mice (db+/db+) have elevated blood glucose, increased or normal insulin levels, and suppressed cell-mediated immunity (Mandel *et al.*, *J. Immunol.* 120:1375 (1978); Debray-Sachs, M. *et al.*, *Clin. Exp. Immunol.* 51(1):1-7 (1983); Leiter *et al.*, *Am. J. of Pathol.* 114:46-55 (1985)). Peripheral neuropathy, myocardial complications, and



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using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

A polypeptide of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups  
5 received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology and immunohistochemistry. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

10 Three groups of 10 animals each (5 diabetic and 5 non-diabetic controls) are evaluated: 1) Vehicle placebo control, 2) untreated group, and 3) treated group.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total square area of the wound. Contraction is then estimated by establishing the differences between the initial wound area (day 0) and that of post  
15 treatment (day 8). The wound area on day 1 is 64mm<sup>2</sup>, the corresponding size of the dermal punch. Calculations are made using the following formula:

$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

20 Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using a Reichert-Jung microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds are used to assess whether the healing process and the morphologic appearance of the repaired skin is altered by  
25 treatment with a polypeptide of the invention. This assessment included verification of the presence of cell accumulation, inflammatory cells, capillaries, fibroblasts, re-epithelialization and epidermal maturity (Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)). A calibrated lens micrometer is used by a blinded observer.

Tissue sections are also stained immunohistochemically with a polyclonal rabbit  
30 anti-human keratin antibody using ABC Elite detection system. Human skin is used as a positive tissue control while non-immune IgG is used as a negative control. Keratinocyte



Proliferating cell nuclear antigen/cyclin (PCNA) in skin specimens is demonstrated by using anti-PCNA antibody (1:50) with an ABC Elite detection system. Human colon cancer can serve as a positive tissue control and human brain tissue can be used as a negative tissue control. Each specimen includes a section with omission of the primary antibody and substitution with non-immune mouse IgG. Ranking of these sections is based on the extent of proliferation on a scale of 0-8, the lower side of the scale reflecting slight proliferation to the higher side reflecting intense proliferation.

### B. Steroid Impaired Rat Model

To demonstrate that a polypeptide of the invention can accelerate the healing process, the effects of multiple topical applications of the polypeptide on full thickness



excisional skin wounds in rats in which healing has been impaired by the systemic administration of methylprednisolone is assessed.

Young adult male Sprague Dawley rats weighing 250-300 g (Charles River Laboratories) are used in this example. The animals are purchased at 8 weeks of age and  
5 are 9 weeks old at the beginning of the study. The healing response of rats is impaired by the systemic administration of methylprednisolone (17mg/kg/rat intramuscularly) at the time of wounding. Animals are individually housed and received food and water *ad libitum*. All manipulations are performed using aseptic techniques. This study is conducted according to the rules and guidelines of Human Genome Sciences, Inc.  
10 Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

The wounding protocol is followed according to section A, above. On the day of wounding, animals are anesthetized with an intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The dorsal region of the animal is shaved and the skin  
15 washed with 70% ethanol and iodine solutions. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is created using a Keyes tissue punch. The wounds are left open for the duration of the experiment. Applications of the testing materials are given topically once a day for 7 consecutive days commencing on the day of wounding and subsequent to methylprednisolone administration. Prior to  
20 treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of wounding and at the end of treatment. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no  
25 longer visible and the wound is covered by a continuous epithelium.

The polypeptide of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for  
30 histology. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.



Four groups of 10 animals each (5 with methylprednisolone and 5 without glucocorticoid) are evaluated: 1) Untreated group 2) Vehicle placebo control 3) treated groups.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total area of the wound. Closure is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is  $64\text{mm}^2$ , the corresponding size of the dermal punch. Calculations are made using the following formula:

$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using an Olympus microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds allows assessment of whether the healing process and the morphologic appearance of the repaired skin is improved by treatment with a polypeptide of the invention. A calibrated lens micrometer is used by a blinded observer to determine the distance of the wound gap.

Experimental data are analyzed using an unpaired t test. A p value of  $< 0.05$  is considered significant.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

### **Example 51: Lymphadema Animal Model**

The purpose of this experimental approach is to create an appropriate and consistent lymphedema model for testing the therapeutic effects of a polypeptide of the invention in lymphangiogenesis and re-establishment of the lymphatic circulatory system in the rat hind limb. Effectiveness is measured by swelling volume of the affected limb, quantification of the amount of lymphatic vasculature, total blood plasma protein, and



histopathology. Acute lymphedema is observed for 7-10 days. Perhaps more importantly, the chronic progress of the edema is followed for up to 3-4 weeks.

Prior to beginning surgery, blood sample is drawn for protein concentration analysis. Male rats weighing approximately ~350g are dosed with Pentobarbital.

Subsequently, the right legs are shaved from knee to hip. The shaved area is swabbed with gauze soaked in 70% EtOH. Blood is drawn for serum total protein testing. Circumference and volumetric measurements are made prior to injecting dye into paws after marking 2 measurement levels (0.5 cm above heel, at mid-pt of dorsal paw). The intradermal dorsum of both right and left paws are injected with 0.05 ml of 1% Evan's Blue. Circumference and volumetric measurements are then made following injection of dye into paws.

Using the knee joint as a landmark, a mid-leg inguinal incision is made circumferentially allowing the femoral vessels to be located. Forceps and hemostats are used to dissect and separate the skin flaps. After locating the femoral vessels, the lymphatic vessel that runs along side and underneath the vessel(s) is located. The main lymphatic vessels in this area are then electrically coagulated suture ligated.

Using a microscope, muscles in back of the leg (near the semitendinosus and adductors) are bluntly dissected. The popliteal lymph node is then located. The 2 proximal and 2 distal lymphatic vessels and distal blood supply of the popliteal node are then and ligated by suturing. The popliteal lymph node, and any accompanying adipose tissue, is then removed by cutting connective tissues.

Care is taken to control any mild bleeding resulting from this procedure. After lymphatics are occluded, the skin flaps are sealed by using liquid skin (Vetbond) (AJ Buck). The separated skin edges are sealed to the underlying muscle tissue while leaving a gap of ~0.5 cm around the leg. Skin also may be anchored by suturing to underlying muscle when necessary.

To avoid infection, animals are housed individually with mesh (no bedding). Recovering animals are checked daily through the optimal edematous peak, which typically occurred by day 5-7. The plateau edematous peak are then observed. To  
30 evaluate the intensity of the lymphedema, the circumference and volumes of 2 designated places on each paw before operation and daily for 7 days are measured. The effect plasma proteins on lymphedema is determined and whether protein analysis is a useful testing



perimeter is also investigated. The weights of both control and edematous limbs are evaluated at 2 places. Analysis is performed in a blind manner.

Circumference Measurements: Under brief gas anesthetic to prevent limb movement, a cloth tape is used to measure limb circumference. Measurements are done at the ankle bone and dorsal paw by 2 different people then those 2 readings are averaged. Readings are taken from both control and edematous limbs.

Volumetric Measurements: On the day of surgery, animals are anesthetized with Pentobarbital and are tested prior to surgery. For daily volumetrics animals are under brief halothane anesthetic (rapid immobilization and quick recovery), both legs are shaved and equally marked using waterproof marker on legs. Legs are first dipped in water, then dipped into instrument to each marked level then measured by Buxco edema software(Chen/Victor). Data is recorded by one person, while the other is dipping the limb to marked area.

Blood-plasma protein measurements: Blood is drawn, spun, and serum separated prior to surgery and then at conclusion for total protein and Ca<sup>2+</sup> comparison.

Limb Weight Comparison: After drawing blood, the animal is prepared for tissue collection. The limbs are amputated using a quillitine, then both experimental and control legs are cut at the ligature and weighed. A second weighing is done as the tibio-cacaneal joint is disarticulated and the foot is weighed.

Histological Preparations: The transverse muscle located behind the knee (popliteal) area is dissected and arranged in a metal mold, filled with freezeGel, dipped into cold methylbutane, placed into labeled sample bags at - 80EC until sectioning. Upon sectioning, the muscle is observed under fluorescent microscopy for lymphatics..

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

### **Example 52: Suppression of TNF alpha-induced adhesion molecule expression by a Polypeptide of the Invention**

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules



(EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

The potential of a polypeptide of the invention to mediate a suppression of TNF- $\alpha$  induced CAM expression can be examined. A modified ELISA assay which uses ECs as a solid phase absorbent is employed to measure the amount of CAM expression on TNF- $\alpha$  treated ECs when co-stimulated with a member of the FGF family of proteins.

HUVECs are seeded in 96-well plates at concentrations of  $1 \times 10^4$  cells/well in EGM medium at 37 degree C for 18-24 hrs or until confluent. The monolayers are subsequently washed 3 times with a serum-free solution of RPMI-1640 supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin, and treated with a given cytokine and/or growth factor(s) for 24 h at 37 degree C. Following incubation, the cells are then evaluated for CAM expression.

Human Umbilical Vein Endothelial cells (HUVECs) are grown in a standard 96 well plate to confluence. Growth medium is removed from the cells and replaced with 90 ul of 199 Medium (10% FBS). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10 ul volumes). Plates are incubated at 37 degree C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are



aspirated to remove medium and 100  $\mu$ l of 0.1% paraformaldehyde-PBS(with  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$ ) is added to each well. Plates are held at 4°C for 30 min.

Fixative is then removed from the wells and wells are washed 1X with PBS(+Ca,Mg)+0.5% BSA and drained. Do not allow the wells to dry. Add 10  $\mu$ l of diluted primary antibody to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10  $\mu$ g/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA.

Then add 20  $\mu$ l of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution) to each well and incubated at 37°C for 30 min. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA. 1 tablet of p-Nitrophenol Phosphate pNPP is dissolved in 5 ml of glycine buffer (pH 10.4). 100  $\mu$ l of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphatase in glycine buffer: 1:5,000 ( $10^0$ ) >  $10^{-0.5}$  >  $10^{-1}$  >  $10^{-1.5}$ . 5  $\mu$ l of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100  $\mu$ l of pNPP reagent must then be added to each of the standard wells. The plate must be incubated at 37°C for 4h. A volume of 50  $\mu$ l of 3M NaOH is added to all wells. The results are quantified on a plate reader at 405 nm. The background subtraction option is used on blank wells filled with glycine buffer only. The template is set up to indicate the concentration of AP-conjugate in each standard well [ 5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

### **Example 53: Assay for the Stimulation of Bone Marrow CD34+ Cell Proliferation**

This assay is based on the ability of human CD34+ to proliferate in the presence of hematopoietic growth factors and evaluates the ability of isolated polypeptides expressed in mammalian cells to stimulate proliferation of CD34+ cells.



It has been previously shown that most mature precursors will respond to only a single signal. More immature precursors require at least two signals to respond. Therefore, to test the effect of polypeptides on hematopoietic activity of a wide range of progenitor cells, the assay contains a given polypeptide in the presence or absence of other hematopoietic growth factors. Isolated cells are cultured for 5 days in the presence of Stem Cell Factor (SCF) in combination with tested sample. SCF alone has a very limited effect on the proliferation of bone marrow (BM) cells, acting in such conditions only as a "survival" factor. However, combined with any factor exhibiting stimulatory effect on these cells (e.g., IL-3), SCF will cause a synergistic effect. Therefore, if the tested polypeptide has a stimulatory effect on a hematopoietic progenitors, such activity can be easily detected. Since normal BM cells have a low level of cycling cells, it is likely that any inhibitory effect of a given polypeptide, or agonists or antagonists thereof, might not be detected. Accordingly, assays for an inhibitory effect on progenitors is preferably tested in cells that are first subjected to *in vitro* stimulation with SCF+IL-3, and then contacted with the compound that is being evaluated for inhibition of such induced proliferation.

Briefly, CD34+ cells are isolated using methods known in the art. The cells are thawed and resuspended in medium (QBSF 60 serum-free medium with 1% L-glutamine (500ml) Quality Biological, Inc., Gaithersburg, MD Cat# 160-204-101). After several gentle centrifugation steps at 200 x g, cells are allowed to rest for one hour. The cell count is adjusted to  $2.5 \times 10^5$  cells/ml. During this time, 100  $\mu$ l of sterile water is added to the peripheral wells of a 96-well plate. The cytokines that can be tested with a given polypeptide in this assay is rhSCF (R&D Systems, Minneapolis, MN, Cat# 255-SC) at 50 ng/ml alone and in combination with rhSCF and rhIL-3 (R&D Systems, Minneapolis, MN, Cat# 203-ML) at 30 ng/ml. After one hour, 10  $\mu$ l of prepared cytokines, 50  $\mu$ l SID (supernatants at 1:2 dilution = 50  $\mu$ l) and 20  $\mu$ l of diluted cells are added to the media which is already present in the wells to allow for a final total volume of 100  $\mu$ l. The plates are then placed in a 37°C/5% CO<sub>2</sub> incubator for five days.

Eighteen hours before the assay is harvested, 0.5  $\mu$ Ci/well of [3H] Thymidine is added in a 10  $\mu$ l volume to each well to determine the proliferation rate. The



experiment is terminated by harvesting the cells from each 96-well plate to a filtermat using the Tomtec Harvester 96. After harvesting, the filtermats are dried, trimmed and placed into OmniFilter assemblies consisting of one OmniFilter plate and one OmniFilter Tray. 60  $\mu$ l Microscint is added to each well and the plate sealed with TopSeal-A press-on sealing film. A bar code 15 sticker is affixed to the first plate for counting. The sealed plates is then loaded and the level of radioactivity determined via the Packard Top Count and the printed data collected for analysis. The level of radioactivity reflects the amount of cell proliferation.

The studies described in this example test the activity of a given polypeptide to stimulate bone marrow CD34+ cell proliferation. One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof. As a nonlimiting example, potential antagonists tested in this assay would be expected to inhibit cell proliferation in the presence of cytokines and/or to increase the inhibition of cell proliferation in the presence of cytokines and a given polypeptide. In contrast, potential agonists tested in this assay would be expected to enhance cell proliferation and/or to decrease the inhibition of cell proliferation in the presence of cytokines and a given polypeptide.

The ability of a gene to stimulate the proliferation of bone marrow CD34+ cells indicates that polynucleotides and polypeptides corresponding to the gene are useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein.

#### **Example 54: Assay for Extracellular Matrix Enhanced Cell Response (EMECCR)**

The objective of the Extracellular Matrix Enhanced Cell Response (EMECCR) assay is to identify gene products (e.g., isolated polypeptides) that act on the hematopoietic stem cells in the context of the extracellular matrix (ECM) induced signal.

Cells respond to the regulatory factors in the context of signal(s) received from the surrounding microenvironment. For example, fibroblasts, and endothelial and epithelial stem cells fail to replicate in the absence of signals from the ECM.



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Additionally, the polynucleotides and/or polypeptides of the gene of interest and/or agonists and/or antagonists thereof, may also be employed to inhibit the proliferation and differentiation of hematopoietic cells and therefore may be employed to protect bone marrow stem cells from chemotherapeutic agents during chemotherapy. This antiproliferative effect may allow administration of higher doses of chemotherapeutic agents and, therefore, more effective chemotherapeutic treatment.

Moreover, polynucleotides and polypeptides corresponding to the gene of interest may also be useful for the treatment and diagnosis of hematopoietic related disorders such as, for example, anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia.

#### **Example 55: Human Dermal Fibroblast and Aortic Smooth Muscle Cell Proliferation**

The polypeptide of interest is added to cultures of normal human dermal fibroblasts (NHDF) and human aortic smooth muscle cells (AoSMC) and two co-assays are performed with each sample. The first assay examines the effect of the polypeptide of interest on the proliferation of normal human dermal fibroblasts (NHDF) or aortic smooth muscle cells (AoSMC). Aberrant growth of fibroblasts or smooth muscle cells is a part of several pathological processes, including fibrosis, and restenosis. The second assay examines IL6 production by both NHDF and SMC. IL6 production is an indication of functional activation. Activated cells will have increased production of a number of cytokines and other factors, which can result in a proinflammatory or immunomodulatory outcome. Assays are run with and without co-TNF $\alpha$  stimulation, in order to check for costimulatory or inhibitory activity.

Briefly, on day 1, 96-well black plates are set up with 1000 cells/well (NHDF) or 2000 cells/well (AoSMC) in 100  $\mu$ l culture media. NHDF culture media contains: Clonetics FB basal media, 1mg/ml hFGF, 5mg/ml insulin, 50mg/ml gentamycin,



2%FBS, while AoSMC culture media contains Clonetics SM basal media, 0.5 µg/ml hEGF, 5mg/ml insulin, 1µg/ml hFGF, 50mg/ml gentamycin, 50 µg/ml Amphotericin B, 5%FBS. After incubation @ 37°C for at least 4-5 hours culture media is aspirated and replaced with growth arrest media. Growth arrest media for NHDF contains  
 5 fibroblast basal media, 50mg/ml gentamycin, 2% FBS, while growth arrest media for AoSMC contains SM basal media, 50mg/ml gentamycin, 50µg/ml Amphotericin B, 0.4% FBS. Incubate at 37C until day 2.

On day 2, serial dilutions and templates of the polypeptide of interest are designed which should always include media controls and known-protein controls.  
 10 For both stimulation and inhibition experiments, proteins are diluted in growth arrest media. For inhibition experiments, TNFa is added to a final concentration of 2ng/ml (NHDF) or 5ng/ml (AoSMC). Then add 1/3 vol media containing controls or supernatants and incubate at 37C/5% CO<sub>2</sub> until day 5.

Transfer 60µl from each well to another labeled 96-well plate, cover with a  
 15 plate-sealer, and store at 4C until Day 6 (for IL6 ELISA). To the remaining 100 µl in the cell culture plate, aseptically add Alamar Blue in an amount equal to 10% of the culture volume (10µl). Return plates to incubator for 3 to 4 hours. Then measure fluorescence with excitation at 530nm and emission at 590nm using the CytoFluor. This yields the growth stimulation/inhibition data.

20 On day 5, the IL6 ELISA is performed by coating a 96 well plate with 50-100 ul/well of Anti-Human IL6 Monoclonal antibody diluted in PBS, pH 7.4, incubate ON at room temperature.

On day 6, empty the plates into the sink and blot on paper towels. Prepare Assay Buffer containing PBS with 4% BSA. Block the plates with 200 µl/well of  
 25 Pierce Super Block blocking buffer in PBS for 1-2 hr and then wash plates with wash buffer (PBS, 0.05% Tween-20). Blot plates on paper towels. Then add 50 µl/well of diluted Anti-Human IL-6 Monoclonal, Biotin-labeled antibody at 0.50 mg/ml. Make dilutions of IL-6 stock in media (30, 10, 3, 1, 0.3, 0 ng/ml). Add duplicate samples to top row of plate. Cover the plates and incubate for 2 hours at RT on shaker.



Wash plates with wash buffer and blot on paper towels. Dilute EU-labeled Streptavidin 1:1000 in Assay buffer, and add 100  $\mu$ l/well. Cover the plate and incubate 1 h at RT. Wash plates with wash buffer. Blot on paper towels.

Add 100  $\mu$ l/well of Enhancement Solution. Shake for 5 minutes. Read the plate on the Wallac DELFIA Fluorometer. Readings from triplicate samples in each assay were tabulated and averaged.

A positive result in this assay suggests AoSMC cell proliferation and that the gene product of interest may be involved in dermal fibroblast proliferation and/or smooth muscle cell proliferation. A positive result also suggests many potential uses of polypeptides, polynucleotides, agonists and/or antagonists of the gene/gene product of interest. For example, inflammation and immune responses, wound healing, and angiogenesis, as detailed throughout this specification. Particularly, polypeptides of the gene product and polynucleotides of the gene may be used in wound healing and dermal regeneration, as well as the promotion of vasculargenesis, both of the blood vessels and lymphatics. The growth of vessels can be used in the treatment of, for example, cardiovascular diseases. Additionally, antagonists of polypeptides of the gene product and polynucleotides of the gene may be useful in treating diseases, disorders, and/or conditions which involve angiogenesis by acting as an anti-vascular (e.g., anti-angiogenesis). These diseases, disorders, and/or conditions are known in the art and/or are described herein, such as, for example, malignancies, solid tumors, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; arteriosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uveitis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophilic joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis. Moreover, antagonists of polypeptides of the



gene product and polynucleotides of the gene may be useful in treating anti-hyperproliferative diseases and/or anti-inflammatory known in the art and/or described herein.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

**Example 56: Cellular Adhesion Molecule (CAM) Expression on Endothelial Cells**

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Briefly, endothelial cells (e.g., Human Umbilical Vein Endothelial cells (HUVECs)) are grown in a standard 96 well plate to confluence, growth medium is removed from the cells and replaced with 100  $\mu$ l of 199 Medium (10% fetal bovine serum (FBS)). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10  $\mu$ l volumes). Plates are then incubated at 37°C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100  $\mu$ l of 0.1% paraformaldehyde-PBS(with Ca<sup>++</sup> and Mg<sup>++</sup>) is added to each well. Plates are held at 4°C for 30 min. Fixative is removed from the wells and wells are washed 1X with PBS(+Ca,Mg) + 0.5% BSA and drained. 10  $\mu$ l of diluted primary antibody is added to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10  $\mu$ g/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are



incubated at 37°C for 30 min. in a humidified environment. Wells are washed three times with PBS(+Ca,Mg) + 0.5% BSA. 20 µl of diluted ExtrAvidin-Alkaline Phosphotase (1:5,000 dilution, referred to herein as the working dilution) are added to each well and incubated at 37°C for 30 min. Wells are washed three times with

5 PBS(+Ca,Mg)+0.5% BSA. Dissolve 1 tablet of p-Nitrophenol Phosphate pNPP per 5 ml of glycine buffer (pH 10.4). 100 µl of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphotase in glycine buffer:  $1:5,000 (10^0) > 10^{-0.5} > 10^{-1} > 10^{-1.5}$ . 5 µl of each dilution is added to triplicate wells and the resulting AP content in

10 each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 µl of pNPP reagent is then added to each of the standard wells. The plate is incubated at 37°C for 4h. A volume of 50 µl of 3M NaOH is added to all wells. The plate is read on a plate reader at 405 nm using the background subtraction option on blank wells filled with glycine buffer only. Additionally, the template is set up to indicate the concentration of AP-

15 conjugate in each standard well [ 5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

#### **Example 57: Alamar Blue Endothelial Cells Proliferation Assay**

This assay may be used to quantitatively determine protein mediated

20 inhibition of bFGF-induced proliferation of Bovine Lymphatic Endothelial Cells (LECs), Bovine Aortic Endothelial Cells (BAECs) or Human Microvascular Uterine Myometrial Cells (UTMECs). This assay incorporates a fluorometric growth indicator based on detection of metabolic activity. A standard Alamar Blue Proliferation Assay is prepared in EGM-2MV with 10 ng /ml of bFGF added as a

25 source of endothelial cell stimulation. This assay may be used with a variety of endothelial cells with slight changes in growth medium and cell concentration. Dilutions of the protein batches to be tested are diluted as appropriate. Serum-free medium (GIBCO SFM) without bFGF is used as a non-stimulated control and Angiostatin or TSP-1 are included as a known inhibitory controls.

30 Briefly, LEC, BAECs or UTMECs are seeded in growth media at a density of 5000 to 2000 cells/well in a 96 well plate and placed at 37-C overnight. After the overnight incubation of the cells, the growth media is removed and replaced with



GIBCO EC-SFM. The cells are treated with the appropriate dilutions of the protein of interest or control protein sample(s) (prepared in SFM ) in triplicate wells with additional bFGF to a concentration of 10 ng/ ml. Once the cells have been treated with the samples, the plate(s) is/are placed back in the 37° C incubator for three days.

- 5 After three days 10 ml of stock alamar blue (Biosource Cat# DAL1100) is added to each well and the plate(s) is/are placed back in the 37°C incubator for four hours. The plate(s) are then read at 530nm excitation and 590nm emission using the CytoFluor fluorescence reader. Direct output is recorded in relative fluorescence units.

- Alamar blue is an oxidation-reduction indicator that both fluoresces and  
 10 changes color in response to chemical reduction of growth medium resulting from cell growth. As cells grow in culture, innate metabolic activity results in a chemical reduction of the immediate surrounding environment. Reduction related to growth causes the indicator to change from oxidized (non-fluorescent blue) form to reduced (fluorescent red) form. i.e. stimulated proliferation will produce a stronger signal and  
 15 inhibited proliferation will produce a weaker signal and the total signal is proportional to the total number of cells as well as their metabolic activity. The background level of activity is observed with the starvation medium alone. This is compared to the output observed from the positive control samples (bFGF in growth medium) and protein dilutions.

20

#### **Example 58: Detection of Inhibition of a Mixed Lymphocyte Reaction**

- This assay can be used to detect and evaluate inhibition of a Mixed Lymphocyte Reaction (MLR) by gene products (e.g., isolated polypeptides). Inhibition of a MLR may be due to a direct effect on cell proliferation and viability,  
 25 modulation of costimulatory molecules on interacting cells, modulation of adhesiveness between lymphocytes and accessory cells, or modulation of cytokine production by accessory cells. Multiple cells may be targeted by these polypeptides since the peripheral blood mononuclear fraction used in this assay includes T, B and natural killer lymphocytes, as well as monocytes and dendritic cells.

- 30 Polypeptides of interest found to inhibit the MLR may find application in diseases associated with lymphocyte and monocyte activation or proliferation. These include, but are not limited to, diseases such as asthma, arthritis, diabetes,



inflammatory skin conditions, psoriasis, eczema, systemic lupus erythematosus, multiple sclerosis, glomerulonephritis, inflammatory bowel disease, crohn's disease, ulcerative colitis, arteriosclerosis, cirrhosis, graft vs. host disease, host vs. graft disease, hepatitis, leukemia and lymphoma.

5 Briefly, PBMCs from human donors are purified by density gradient centrifugation using Lymphocyte Separation Medium (LSM<sup>®</sup>, density 1.0770 g/ml, Organon Teknika Corporation, West Chester, PA). PBMCs from two donors are adjusted to  $2 \times 10^6$  cells/ml in RPMI-1640 (Life Technologies, Grand Island, NY) supplemented with 10% FCS and 2 mM glutamine. PBMCs from a third donor is  
10 adjusted to  $2 \times 10^5$  cells/ml. Fifty microliters of PBMCs from each donor is added to wells of a 96-well round bottom microtiter plate. Dilutions of test materials (50  $\mu$ l) is added in triplicate to microtiter wells. Test samples (of the protein of interest) are added for final dilution of 1:4; rhuIL-2 (R&D Systems, Minneapolis, MN, catalog number 202-IL) is added to a final concentration of 1  $\mu$ g/ml; anti-CD4 mAb (R&D  
15 Systems, clone 34930.11, catalog number MAB379) is added to a final concentration of 10  $\mu$ g/ml. Cells are cultured for 7-8 days at 37°C in 5% CO<sub>2</sub>, and 1  $\mu$ C of [<sup>3</sup>H] thymidine is added to wells for the last 16 hrs of culture. Cells are harvested and thymidine incorporation determined using a Packard TopCount. Data is expressed as the mean and standard deviation of triplicate determinations.

20 Samples of the protein of interest are screened in separate experiments and compared to the negative control treatment, anti-CD4 mAb, which inhibits proliferation of lymphocytes and the positive control treatment, IL-2 (either as recombinant material or supernatant), which enhances proliferation of lymphocytes.

25 One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above  
30 teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other



5



***What Is Claimed Is:***

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:
- (a) a polynucleotide fragment of SEQ ID NO:X or a polynucleotide fragment of the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
  - (b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:Y or a polypeptide fragment encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
  - (c) a polynucleotide encoding a polypeptide domain of SEQ ID NO:Y or a polypeptide domain encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
  - (d) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:Y or a polypeptide epitope encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
  - (e) a polynucleotide encoding a polypeptide of SEQ ID NO:Y or the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X, having biological activity;
  - (f) a polynucleotide which is a variant of SEQ ID NO:X;
  - (g) a polynucleotide which is an allelic variant of SEQ ID NO:X;
  - (h) a polynucleotide which encodes a species homologue of the SEQ ID NO:Y;
  - (i) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues.



5           3.       The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:Y or the polypeptide encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X.

15            5.        The isolated nucleic acid molecule of claim 2, wherein the nucleotide  
sequence comprises sequential nucleotide deletions from either the C-terminus or the  
N-terminus.

7. A recombinant vector comprising the isolated nucleic acid molecule of claim 1.

9. A recombinant host cell produced by the method of claim 8.

30

10. The recombinant host cell of claim 9 comprising vector sequences.



11. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a polypeptide fragment of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

5 (b) a polypeptide fragment of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z, having biological activity;

(c) a polypeptide domain of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

10 (d) a polypeptide epitope of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

(e) a secreted form of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

(f) a full length protein of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;

15 (g) a variant of SEQ ID NO:Y;

(h) an allelic variant of SEQ ID NO:Y; or

(i) a species homologue of the SEQ ID NO:Y.

20 12. The isolated polypeptide of claim 11, wherein the secreted form or the full length protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.

13. An isolated antibody that binds specifically to the isolated polypeptide of claim 11.

25 14. A recombinant host cell that expresses the isolated polypeptide of claim 11.

15. A method of making an isolated polypeptide comprising:

30 (a) culturing the recombinant host cell of claim 14 under conditions such that said polypeptide is expressed; and

(b) recovering said polypeptide.







- (c) detecting an activity in a biological assay; and
- (d) identifying the protein in the supernatant having the activity.

23. The product produced by the method of claim 20.



## 5

10



## DECLARATION FOR PATENT APPLICATION

As a below named inventor, I declare that I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

### **27 HUMAN SECRETED PROTEINS**

the specification of which is being filed concurrently herewith.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application, which designated at least one country other than the United States listed below, and have also identified below any foreign application for patent or inventor's certificate, or PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s):

Priority Claimed

Yes No

\_\_\_\_\_  
(Number)

\_\_\_\_\_  
(Country)

\_\_\_\_\_  
(Day/Month/Year Filed)

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below.

60/125,055

(Application Serial No.)

18 March 1999

(Filing Date)

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or under § 365(b) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information that is material to patentability as defined in 37 C.F.R. § 1.56 that became available between the filing date of the prior application and the national or PCT international filing date of this application.

PCT/US00/06783

(Application Serial No.)

16 March 2000

(Filing Date)

Pending

(Status: patented, pending, abandoned)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: James H. Davis (Reg. No. 40,582), Kenley Hoover (Reg. No. 40,302), Joseph Kenny (Reg. No. 43,710), Jonathan L. Klein (Reg. No. 41,119), and Michele Wales (Reg. No. 43,975) of Human Genome Sciences, Inc. 9410 Key West Avenue, Rockville, Maryland, 20878.



I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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 <212> DNA  
 <213> Homo sapiens

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 <212> DNA  
 <213> Homo sapiens

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 <223> n equals a,t,g, or c

<220>  
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<220>  
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<211> 2229
<212> DNA
<213> Homo sapiens

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<223> n equals a,t,g, or c

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&lt;211&gt; 2105

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 23

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&lt;211&gt; 1290

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 24

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<223> n equals a,t,g, or c

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<212> DNA
<213> Homo sapiens

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```

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<210> 32
<211> 2287
<212> DNA
<213> Homo sapiens

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```

<220>
<221> SITE
<222> (1370)
<223> n equals a,t,g, or c

```

```

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```



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<210> 33
<211> 688
<212> DNA
<213> Homo sapiens
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<211> 995
<212> DNA
<213> Homo sapiens
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<211> 765
<212> DNA
<213> Homo sapiens
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<222> (680)
<223> n equals a,t,g, or c
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<221> SITE
<222> (683)
<223> n equals a,t,g, or c
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<221> SITE
<222> (699)
<223> n equals a,t,g, or c
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```
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<221> SITE
<222> (740)
<223> n equals a,t,g, or c
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<220>
<221> SITE
<222> (765)
<223> n equals a,t,g, or c
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[illegible]



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<212> DNA
<213> Homo sapiens
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<211> 5065
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<210> 41  
 <211> 2248  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (2234)  
 <223> n equals a,t,g, or c

<220>  
 <221> SITE  
 <222> (2248)  
 <223> n equals a,t,g, or c

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<210> 42



<211> 1037  
 <212> DNA  
 <213> Homo sapiens  
 <220>  
 <221> SITE  
 <222> (12)  
 <223> n equals a,t,g, or c

<400> 42  
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 ccgagggggc ggcgct 1037

<210> 43  
 <211> 2102  
 <212> DNA  
 <213> Homo sapiens

<400> 43  
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 ccatcgtggc gttctgctgt gcccgttccc agagaaatct caaagggtgt gtgtcagcca 240  
 aaaatgatat ccgagtggaa attgtccaca aggaaccagc ctctggtcgg gaggggtgagg 300  
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at 2102

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<210> 44
<211> 1362
<212> DNA
<213> Homo sapiens

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<400> 44
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aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaagggcgg cc 1362

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<210> 45
<211> 390
<212> DNA
<213> Homo sapiens

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<400> 45
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cccagctcc caggcctctt ggccctgaca tctttttata aaccaggaca gtgtttagga 300
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gctctcgctt aaaaaaaaaa aaaaaaaaaa

```

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<210> 46
<211> 1546
<212> DNA
<213> Homo sapiens

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```

<400> 46
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tatagcatgg acctcccaga gaagctcaag ctatgtggca ctgtagcttt gccgtgaatg 360

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```

<210> 47
<211> 1643
<212> DNA
<213> Homo sapiens

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<220>
<221> SITE
<222> (5)
<223> n equals a,t,g, or c

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```

<220>
<221> SITE
<222> (59)
<223> n equals a,t,g, or c

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<400> 47
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ctgacggggc tctcagggtc gggaggacc cagtgccttc ctcagaagaa gcacatgggc 360
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ctctcttggc ctggaagaat aacactgaaa aaacctgacg ctgcggtcac ttagcagagg 540
ctcaggttct tgccttggga aacactacta gctctgacct tccataacct acttggggga 600
gcacagggcc ccgctggctt cctcaccaac ggcagtgcga aaatcagccc ccacatcaag 660
gtggtgttct ctgtgcttct tctgtcctt ccaaagtcgg ttctggccta acgcatgtcc 720
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cacatttaaa aatctccgtg ttgcttttaa aaatgggttt ttgttttttt gtttttttga 960
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acttaagtca aggaggttg aaattcacag gccaggggc acctttttatt tttttcata 1560
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aaaaaaaaa aaaaaaactc gag 1643

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<220>
<221> SITE
<222> (1)
<223> n equals a,t,g, or c
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<210> 49
<211> 1093
<212> DNA
<213> Homo sapiens
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<210> 50
<211> 2752
<212> DNA
<213> Homo sapiens
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[illegible]



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aaatgttccc gaatgcattt gcacaaattc agctgctatg acaattttgt gaaaagtaac 660
acgttttggtg cccagaaaca caatactgac tttggagctt ttaagcacct ttatatgtta 720
ttagttaatg cttttaattt aagtcagaat agtttatcaa agaaaagttt gaatgtttgg 780
aataaggact ccatagcatc taactgtaga tccagtcctt ctcatactac gaatggttgc 840
cagggagaaa aagtgaggac ctgtgaaaaa tctgatgagt ctgccatgag tttttatcca 900
ccatcactaa atgacgcac ttttactttg attggattca gtaaagggtt tggtgttttg 960
aatcagttgc tttttgaatt gaaagaagcc aagaaagaca agaacataga tgctttttatc 1020
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<210> 51
<211> 761
<212> PRT
<213> Homo sapiens

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<220>
<221> SITE
<222> (376)
<223> Xaa equals any of the naturally occurring L-amino acids

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<220>
<221> SITE
<222> (380)
<223> Xaa equals any of the naturally occurring L-amino acids

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<220>
<221> SITE
<222> (381)
<223> Xaa equals any of the naturally occurring L-amino acids

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<220>
<221> SITE
<222> (384)
<223> Xaa equals any of the naturally occurring L-amino acids

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His Gly Pro Ala Ala Val Pro Glu Ala Ser Ser Thr Val Tyr Asn Gly  
 595 600 605  
 Ser Leu Leu Leu Ile Val Gln Asp Gly Val Gly Gly Leu Tyr Gln Cys  
 610 615 620  
 Trp Ala Thr Glu Asn Gly Phe Ser Tyr Pro Val Ile Ser Tyr Trp Val  
 625 630 635 640  
 Asp Ser Gln Asp Gln Thr Leu Ala Leu Asp Pro Glu Leu Ala Gly Ile  
 645 650 655  
 Pro Arg Glu His Val Lys Val Pro Leu Thr Arg Val Ser Gly Gly Ala  
 660 665 670  
 Ala Leu Ala Ala Gln Gln Ser Tyr Trp Pro His Phe Val Thr Val Thr  
 675 680 685  
 Val Leu Phe Ala Leu Val Leu Ser Gly Ala Leu Ile Ile Leu Val Ala  
 690 695 700  
 Ser Pro Leu Arg Ala Leu Arg Ala Arg Gly Lys Val Gln Gly Cys Glu  
 705 710 715 720  
 Thr Leu Arg Pro Gly Glu Lys Ala Pro Leu Ser Arg Glu Gln His Leu  
 725 730 735  
 Gln Ser Pro Lys Glu Cys Arg Thr Ser Ala Ser Asp Val Asp Ala Asp  
 740 745 750  
 Asn Asn Cys Leu Gly Thr Glu Val Ala  
 755 760  
  
 <210> 52  
 <211> 305  
 <212> PRT  
 <213> Homo sapiens  
  
 <400> 52  
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 20 25 30  
 Lys Leu Tyr Ala Glu Asn Leu Pro Cys Val His Leu Asn Pro Gln Trp  
 35 40 45  
 Pro Ser Gln Pro Ser His Cys Pro Arg Gly Trp Arg Ser Asn Pro Leu  
 50 55 60  
 Pro Pro Ala Ala Gly His Ser Arg Ala Gln Glu Asp Lys Val Leu Gly  
 65 70 75 80  
 Gly His Glu Cys Gln Pro His Ser Gln Pro Trp Gln Ala Ala Leu Phe  
 85 90 95  
 Gln Gly Gln Gln Leu Leu Cys Gly Gly Val Leu Val Gly Gly Asn Trp  
 100 105 110  
 Val Leu Thr Ala Ala His Cys Lys Lys Pro Lys Tyr Thr Val Arg Leu  
 115 120 125  
 Gly Asp His Ser Leu Gln Asn Lys Asp Gly Pro Glu Gln Glu Ile Pro  
 130 135 140







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<210> 54
<211> 228
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (207)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (217)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (218)
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<400> 54

Ala Ser Ala Ser Tyr Ala Leu Gly Asn Thr Pro Arg His Arg Gln Ser  
20 25 30

Leu Pro Arg Pro Ser Gly Gln Thr Ser Val Thr Thr Ser Cys Cys Asn  
35 40 45

Leu Leu Thr Glu Leu Arg His Pro Ser Ser Ala Asp Phe Gly His Gln  
50 55 60

Ser Ser Arg Phe Ser Leu Leu Glu Leu Arg His Pro Ser Ala Ala Ala  
65 70 75 80

Cys Gly His Gln Asn Ser Arg Phe Ser Leu Leu Glu Leu Arg Arg Pro  
85 90 95

Ser Ser Asp Ala Phe Gly His Gln Ser Ser Arg Leu Ser Leu Leu Asp  
100 105 110

Leu Arg His Thr Ser Ala Ala Phe Gly His Gln Asn Ser Arg Phe  
115 120 125

Ser Leu Val Glu Leu Arg His Pro Ser Ser Asp Ala Phe Gly His Gln  
130 135 140

Asn Ser Arg Phe Cys Phe Leu Asp Leu Arg His Pro Ser Ala Ala Ala  
145 150 155 160

Phe Gly His Gln Asn Ser Arg Phe Ser His Val Glu Pro Arg His Pro  
165 170 175

Ser Ser Ala Ala Phe Gly His Gln Asn Ser Arg Phe Ser Gly Leu Cys  
180 185 190

Thr Leu Gly Cys Val Ala Ala Thr Pro Ala Pro Gly Phe Gln Xaa Phe  
195 200 205

Gly Leu Arg Leu Gln Ala Thr Pro Xaa Xaa Ser Leu Val Leu Arg Leu  
210 215 220

Leu Asp Leu Asp  
225

<210> 55

<211> 552

&lt;212&gt; PRT

<213> Homo sapiens

<400> 55

Met Leu Lys Ala Ser Cys Leu Pro Leu Gly Phe Ile Val Phe Leu Pro  
1 5 10 15

Ala Val Leu Leu Leu Val Ala Pro Pro Leu Pro Ala Ala Asp Ala Ala  
20 25 30

His Glu Phe Thr Val Tyr Arg Met Gln Gln Tyr Asp Leu Gln Gly Gln  
35 40 45

Pro Tyr Gly Thr Arg Asn Ala Val Leu Asn Thr Glu Ala Arg Thr Met  
50 55 60



Ala Ala Glu Val Leu Ser Arg Arg Cys Val Leu Met Arg Leu Leu Asp  
 65 70 75 80  
 Phe Ser Tyr Glu Gln Tyr Gln Lys Ala Leu Arg Gln Ser Ala Gly Ala  
 85 90 95  
 Val Val Ile Ile Leu Pro Arg Ala Met Ala Ala Val Pro Gln Asp Val  
 100 105 110  
 Val Arg Gln Phe Met Glu Ile Glu Pro Glu Met Leu Ala Met Glu Thr  
 115 120 125  
 Ala Val Pro Val Tyr Phe Ala Val Glu Asp Glu Ala Leu Leu Ser Ile  
 130 135 140  
 Tyr Lys Gln Thr Gln Ala Ala Ser Ala Ser Gln Gly Ser Ala Ser Ala  
 145 150 155 160  
 Ala Glu Val Leu Leu Arg Thr Ala Thr Ala Asn Gly Phe Gln Met Val  
 165 170 175  
 Thr Ser Gly Val Gln Ser Lys Ala Val Ser Asp Trp Leu Ile Ala Ser  
 180 185 190  
 Val Glu Gly Arg Leu Thr Gly Leu Gly Gly Glu Asp Leu Pro Thr Ile  
 195 200 205  
 Val Ile Val Ala His Tyr Asp Ala Phe Gly Val Ala Pro Trp Leu Ser  
 210 215 220  
 Leu Gly Ala Asp Ser Asn Gly Ser Gly Val Ser Val Leu Leu Glu Leu  
 225 230 235 240  
 Ala Arg Leu Phe Ser Arg Leu Tyr Thr Tyr Lys Arg Thr His Ala Ala  
 245 250 255  
 Tyr Asn Leu Leu Phe Phe Ala Ser Gly Gly Gly Lys Phe Asn Tyr Gln  
 260 265 270  
 Gly Thr Lys Arg Trp Leu Glu Asp Asn Leu Asp His Thr Asp Ser Ser  
 275 280 285  
 Leu Leu Gln Asp Asn Val Ala Phe Val Leu Cys Leu Asp Thr Val Gly  
 290 295 300  
 Arg Gly Ser Ser Leu His Leu His Val Ser Lys Pro Pro Arg Glu Gly  
 305 310 315 320  
 Thr Leu Gln His Ala Phe Leu Arg Glu Leu Glu Thr Val Ala Ala His  
 325 330 335  
 Gln Phe Pro Glu Val Arg Phe Ser Met Val His Lys Arg Ile Asn Leu  
 340 345 350  
 Ala Glu Asp Val Leu Ala Trp Glu His Glu Arg Phe Ala Ile Arg Arg  
 355 360 365  
 Leu Pro Ala Phe Thr Leu Ser His Leu Glu Ser His Arg Asp Gly Gln  
 370 375 380  
 Arg Ser Ser Ile Met Asp Val Arg Ser Arg Val Asp Ser Lys Thr Leu  
 385 390 395 400  
 Thr Arg Asn Thr Arg Ile Ile Ala Glu Ala Leu Thr Arg Val Ile Tyr  
 405 410 415



Asn	Leu	Thr	Glu	Lys	Gly	Thr	Pro	Asp	Met	Pro	Val	Phe	Thr	Glu
			420					425				430		
Gln	Met	Gln	Ile	Gln	Gln	Glu	Gln	Leu	Asp	Ser	Val	Met	Asp	Trp
		435					440					445		Leu
Thr	Asn	Gln	Pro	Arg	Ala	Ala	Gln	Leu	Val	Asp	Lys	Asp	Ser	Thr
	450					455					460			Phe
Leu	Ser	Thr	Leu	Glu	His	His	Leu	Ser	Arg	Tyr	Leu	Lys	Asp	Val
465					470					475				Lys
Gln	His	His	Val	Lys	Ala	Asp	Lys	Arg	Asp	Pro	Glu	Phe	Val	Phe
				485					490					495
Asp	Gln	Leu	Lys	Gln	Val	Met	Asn	Ala	Tyr	Arg	Val	Lys	Pro	Ala
			500					505					510	Val
Phe	Asp	Leu	Leu	Leu	Ala	Val	Gly	Ile	Ala	Ala	Tyr	Leu	Gly	Met
		515					520					525		Ala
Tyr	Val	Ala	Val	Gln	His	Phe	Ser	Leu	Leu	Tyr	Lys	Thr	Val	Gln
	530					535					540			Arg
Leu	Leu	Val	Lys	Ala	Lys	Thr	Gln							
545					550									
<210> 56														
<211> 385														
<212> PRT														
<213> Homo sapiens														
<400> 56														
Met	Ser	Phe	Ile	Met	Lys	Leu	His	Arg	His	Phe	Gln	Arg	Thr	Val
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Leu	Leu	Ala	Thr	Phe	Cys	Met	Val	Ser	Ile	Ile	Ile	Ser	Ala	Tyr
			20					25					30	Tyr
Leu	Tyr	Ser	Gly	Tyr	Lys	Gln	Glu	Asn	Glu	Leu	Ser	Glu	Thr	Ala
		35					40					45		Ser
Glu	Val	Asp	Cys	Gly	Asp	Leu	Gln	His	Leu	Pro	Tyr	Gln	Leu	Met
	50					55					60			Glu
Val	Lys	Ala	Met	Lys	Leu	Phe	Asp	Ala	Ser	Arg	Thr	Asp	Pro	Thr
65					70					75				80
Leu	Val	Phe	Val	Glu	Ser	Gln	Tyr	Ser	Ser	Leu	Gly	Gln	Asp	Ile
				85					90					95
Met	Ile	Leu	Glu	Ser	Ser	Arg	Phe	Gln	Tyr	His	Ile	Glu	Ile	Ala
			100					105					110	Pro
Gly	Lys	Gly	Asp	Leu	Pro	Val	Leu	Ile	Asp	Lys	Met	Lys	Gly	Lys
		115					120					125		Tyr
Ile	Leu	Ile	Ile	Tyr	Glu	Asn	Ile	Leu	Lys	Tyr	Ile	Asn	Met	Asp
	130					135					140			Ser
Trp	Asn	Arg	Ser	Leu	Leu	Asp	Lys	Tyr	Cys	Val	Glu	Tyr	Gly	Val
145					150					155				Gly
Val	Ile	Gly	Phe	His	Lys	Thr	Ser	Glu	Lys	Ser	Val	Gln		



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<210> 57
<211> 190
<212> PRT
<213> Homo sapiens
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<220>
<221> SITE
<222> (180)
<223> Xaa equals any of the naturally occurring L-amino acids
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Val Leu Ala Glu Arg Leu Phe Arg Arg Ala Leu Arg Pro Asp Pro Ser  
20 25 30







Pro Ala Arg Glu Arg Arg Gly Arg Glu Gln Ser Thr Leu Glu Ser Glu  
 50 55 60  
 Glu Trp Asn Pro Trp Glu Gly Asp Glu Lys Asn Glu Gln Gln His Arg  
 65 70 75 80  
 Phe Lys Thr Ser Leu Gln Ile Leu Asp Lys Ser Thr Lys Gly Lys Thr  
 85 90 95  
 Asp Leu Ser Val Gln Ile Trp Gly Lys Ala Ala Ile Gly Leu Tyr Leu  
 100 105 110  
 Trp Glu His Ile Phe Glu Gly Leu Leu Asp Pro Ser Asp Val Thr Ala  
 115 120 125  
 Gln Trp Arg Glu Gly Lys Ser Ile Val Gly Arg Thr Gln Tyr Ser Phe  
 130 135 140  
 Ile Thr Gly Pro Ala Val Ile Pro Gly Tyr Phe Ser Val Asp Val Asn  
 145 150 155 160  
 Asn Val Val Leu Ile Leu Asn Gly Arg Glu Lys Ala Lys Ile Phe Tyr  
 165 170 175  
 Ala Thr Gln Trp Leu Leu Tyr Ala Gln Asn Leu Val Gln Ile Gln Lys  
 180 185 190  
 Leu Gln His Leu Ala Val Val Leu Leu Gly Asn Glu His Cys Asp Asn  
 195 200 205  
 Glu Trp Ile Asn Pro Phe Leu Lys Arg Asn Gly Gly Phe Val Glu Leu  
 210 215 220  
 Leu Phe Ile Ile Tyr Asp Ser Pro Trp Ile Asn Asp Val Asp Val Phe  
 225 230 235 240  
 Gln Trp Pro Leu Gly Val Ala Thr Tyr Arg Asn Phe Pro Val Val Glu  
 245 250 255  
 Ala Ser Trp Ser Met Leu His Asp Glu Arg Pro Tyr Leu Cys Asn Phe  
 260 265 270  
 Leu Gly Thr Ile Tyr Glu Asn Ser Ser Arg Gln Ala Leu Met Asn Ile  
 275 280 285  
 Leu Lys Lys Asp Gly Asn Asp Lys Leu Cys Trp Val Ser Ala Arg Glu  
 290 295 300  
 His Trp Gln Pro Gln Glu Thr Asn Glu Ser Leu Lys Asn Tyr Gln Asp  
 305 310 315 320  
 Ala Leu Leu Gln Ser Asp Leu Thr Leu Cys Pro Val Gly Val Asn Thr  
 325 330 335  
 Glu Cys Tyr Arg Ile Tyr Glu Ala Cys Ser Tyr Gly Ser Ile Pro Val  
 340 345 350  
 Val Glu Asp Val Met Thr Ala Gly Asn Cys Gly Asn Thr Ser Val His  
 355 360 365  
 His Gly Ala Pro Leu Gln Leu Leu Lys Ser Met Gly Ala Pro Phe Ile  
 370 375 380  
 Phe Ile Lys Asn Trp Lys Glu Leu Pro Ala Val Leu Glu Lys Glu Lys  
 385 390 395 400



<220>  
<221> SITE



&lt;222&gt; (137)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;400&gt; 61

Met Leu Leu Phe Asn Trp Ile Cys Ile Val Ile Thr Gly Leu Ala Met  
 1 5 10 15

Asp Met Gln Leu Leu Met Ile Pro Leu Ile Met Ser Val Leu Tyr Val  
 20 25 30

Trp Ala Gln Leu Asn Arg Asp Met Ile Val Ser Phe Trp Phe Gly Thr  
 35 40 45

Arg Phe Lys Ala Cys Tyr Leu Pro Trp Val Ile Leu Gly Phe Asn Tyr  
 50 55 60

Ile Ile Gly Gly Ser Val Ile Asn Glu Leu Ile Gly Asn Leu Val Gly  
 65 70 75 80

His Leu Tyr Phe Phe Leu Met Phe Arg Tyr Pro Met Asp Leu Gly Gly  
 85 90 95

Arg Asn Phe Leu Ser Thr Pro Gln Phe Leu Tyr Arg Trp Leu Pro Ser  
 100 105 110

Arg Arg Gly Gly Val Ser Gly Phe Gly Val Pro Pro Ala Ser Met Arg  
 115 120 125

Arg Ala Ala Asp Gln Asn Gly Gly Xaa Gly Arg His Asn Trp Gly Gln  
 130 135 140

Gly Phe Arg Leu Gly Asp Gln  
 145 150

&lt;210&gt; 62

&lt;211&gt; 118

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 62

Met Ser Arg Ser Val Ala Leu Ala Val Leu Ala Leu Leu Ser Leu Ser  
 1 5 10 15

Gly Leu Glu Ala Ile Gln Arg Glu Ser Ser Pro Thr Leu Pro Ala Leu  
 20 25 30

Val Leu Pro Leu Pro Leu Cys Thr Leu Cys Gly Pro Arg Cys Ala Leu  
 35 40 45

Ser Leu Arg Asp Phe Pro Ser Pro Ser Ser Pro Trp Trp Pro Ala Val  
 50 55 60

Gly Leu Val Gln Gly Trp Ile Ser Gly Lys Arg Arg Gly Gly Leu Gly  
 65 70 75 80

Val Gly Lys Gly Val Arg Thr Arg Asp Ala Arg Tyr Leu Pro Leu Ser  
 85 90 95

Ala Gly Ser Arg Gly Asp Leu Trp Pro Thr Ala Thr Gly Gly Ser Gly  
 100 105 110

Gln Ser Leu Gly Arg Arg  
 115

090454950



<400>	63															
Met	Ala	Val	Ile	Ile	Gly	Val	Ala	Val	Gly	Ala	Gly	Val	Ala	Phe	Leu	
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Val	Leu	Met	Ala	Thr	Ile	Val	Ala	Phe	Cys	Cys	Ala	Arg	Ser	Gln	Arg	
			20					25					30			
Asn	Leu	Lys	Gly	Val	Val	Ser	Ala	Lys	Asn	Asp	Ile	Arg	Val	Glu	Ile	
		35					40					45				
Val	His	Lys	Glu	Pro	Ala	Ser	Gly	Arg	Glu	Gly	Glu	Glu	His	Ser	Thr	
	50					55					60					
Ile	Lys	Gln	Leu	Met	Met	Asp	Arg	Gly	Glu	Phe	Gln	Gln	Asp	Ser	Val	
65					70					75					80	
Leu	Lys	Gln	Leu	Glu	Val	Leu	Lys	Glu	Glu	Glu	Lys	Glu	Phe	Gln	Asn	
				85					90					95		
Leu	Lys	Asp	Pro	Thr	Asn	Gly	Tyr	Tyr	Ser	Val	Asn	Thr	Phe	Lys	Glu	
			100					105					110			
His	His	Ser	Thr	Pro	Thr	Ile	Ser	Leu	Ser	Ser	Cys	Gln	Pro	Asp	Leu	
		115					120					125				
Arg	Pro	Ala	Gly	Lys	Gln	Arg	Val	Pro	Thr	Gly	Met	Ser	Phe	Thr	Asn	
	130					135					140					
Ile	Tyr	Ser	Thr	Leu	Ser	Gly	Gln	Gly	Arg	Leu	Tyr	Asp	Tyr	Gly	Ser	
145					150					155					160	
Gly	Leu	Cys	Trp	Ala	Trp	Ala	Ala	Arg	Pro	Ser	Ser	Phe	Val	Ser	Gly	
				165					170					175		
Ser	Ser	Arg	Glu	Ala	Pro	Ser	Ala	Thr	Ala	Ala	Pro	Ser	Trp	Thr	Arg	
			180					185					190			
Ser	Val	Thr	Ala	Ala	Ser	Ala	Ala	Ala	Ala	Ser	Arg	Met	Ala	Met	Cys	
		195					200					205				
Ser	Ser	Thr	Arg	Pro	Ala	Arg	Leu	Leu	Leu	Pro	Pro	Pro	Thr	Thr	Pro	
	210					215					220					
Ser	Pro	Arg	Pro	Arg	Thr	Leu	Thr	Pro	Val	Asp	Pro	Cys	Ser	Gly	Gly	
225					230					235					240	
Cys	Arg	Leu	Thr	Ser	Lys	Asp	His	Thr	Pro	Arg	Val	Gly	Thr	Gly	Gln	
				245					250					255		
Gly	Arg	Gly	Gln	Gly	Thr	Phe	Trp	Leu	Ser	Arg	Asp	Glu	Gly	Tyr	Phe	
			260					265					270			
Ala	Glu	Asp	Thr	Arg	Ile	Gly	His	Phe	Gln	Asp	Ser	Leu	Pro	Ala	Pro	
		275					280					285				
Leu	Pro	Leu	Pro	Ser	Phe	Glu	Ala	Leu	Ile	Lys	His	Lys	Ser	Gly	Ser	
	290					295					300					
Pro	Gly	Ala	Val	Cys	Gln	Arg	Trp	Ala	Gly	Gly	Glu	Thr	Asp	Arg	Gly	
305					310					315					320	



Cys Gly

<210> 64  
 <211> 41  
 <212> PRT  
 <213> Homo sapiens

<400> 64  
 Met Ala Gln Cys Cys Leu Trp Leu Gly Ser Trp Val Leu Asp Met Ala  
           1                          5                          10                          15  
 Ser Cys Ser Pro Phe Ser Thr Gly Ile Trp Lys Thr Ser Met Glu Leu  
                           20                          25                          30  
 Gln Pro Ser Leu Gly Ser Val Gln Ser  
                           35                          40

<210> 65  
 <211> 152  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (73)  
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>  
 <221> SITE  
 <222> (106)  
 <223> Xaa equals any of the naturally occurring L-amino acids

<400> 65  
 Met Arg Thr Cys Gly Ile Trp Phe Cys Phe Cys Thr Ser Ser Leu Arg  
           1                          5                          10                          15  
 Ile Met Ala Ser Ser Phe Thr Tyr Val Ala Ala Lys Asn Met Ile Ser  
                           20                          25                          30  
 Leu Leu Leu Trp Leu His Ser Glu Met Gly Lys Val Pro Leu Ser Pro  
                           35                          40                          45  
 Ser Gln Gly Val Arg Trp Gly Cys Asp Ser Leu Leu Gln Cys Pro Ala  
           50                          55                          60  
 Ala Gln Thr Ser Met Gly Gly Met Xaa Thr Gly Arg Leu Trp Gly Ser  
           65                          70                          75                          80  
 Asp Pro Lys Ala Val Ser Arg Gly Glu Ala Pro Val Gly Val Cys Tyr  
                           85                          90                          95  
 Arg Val Leu Phe Gln Phe Ser Arg Pro Xaa Ala Ala Cys Val Leu Ser  
                           100                          105                          110  
 Ser Ile Arg Pro Leu Pro Tyr Arg Lys Asp Arg Gly Leu Ser Val Ser  
           115                          120                          125  
 Leu Gly Ser Cys Leu Gly Val Leu Glu Glu Ser Asp His Thr Trp Ala  
           130                          135                          140  
 Trp Arg Leu Ser Thr Arg Phe Cys  
           145                          150



```

<400> 68
Met Thr Leu Ile Glu Gly Val Gly Asp Glu Val Thr Val Leu Phe Ser
  1          5          10          15
Val Leu Ala Cys Leu Leu Val Leu Ala Leu Ala Trp Val Ser Thr His
          20          25          30
Thr Ala Glu Gly Gly Asp Pro Leu Pro Gln Pro Ser Gly Thr Pro Thr
          35          40          45
Pro Ser Gln Pro Ser Ala Ala Met Ala Ala Thr Asp Ser Met Arg Gly
  50          55          60

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Met Ala Ser Leu Arg Ser Gln His Gly Pro Gly Ala Pro Glu Ser Leu



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<210> 70
<211> 90
<212> PRT
<213> Homo sapiens
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<210> 71
<211> 43
<212> PRT
<213> Homo sapiens
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<210> 72
<211> 53
<212> PRT
<213> Homo sapiens
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&lt;400&gt; 72

Met Leu Met Arg Tyr Lys Ser Tyr Phe Phe Ile Ser Ile Leu Leu Leu  
 1 5 10 15

Cys Cys Phe Phe Phe Leu Ile Leu Gln Val Tyr Lys Leu Ser Phe Lys  
 20 25 30

Ile Leu Ser Gln Asp Phe Lys Asn Cys Arg Val Leu Val Trp Arg Ser  
 35 40 45

Leu Pro Ser Phe Ser  
 50

&lt;210&gt; 73

&lt;211&gt; 105

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 73

Met Ser Phe Leu Gly Phe Ile Leu Asn Leu Gly Ala Arg Leu Ile Val  
 1 5 10 15

Gln Pro Gln Ala Ala Leu Ala Ser Arg Gly Leu Arg Gly Gln Gly Leu  
 20 25 30

Pro Cys Glu Thr Gln Val Cys Lys Arg Thr Leu Arg Pro Gly Ala Val  
 35 40 45

Gly Trp Leu Val His Lys Gly Arg Arg Ala Leu Ser Ile Ser Arg Lys  
 50 55 60

Ser Ala Leu Val Ser Leu Gly Val Met Tyr Val Gly Pro Gly Lys Arg  
 65 70 75 80

Pro Gly Val Val Arg Lys His Ser Leu Leu Val Lys Met Gln Ala Arg  
 85 90 95

Gly Lys Glu Val Ser Pro Thr Met Cys  
 100 105

&lt;210&gt; 74

&lt;211&gt; 192

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (48)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (49)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (78)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;400&gt; 74

Met Trp Leu Leu Cys Val Ala Leu Ala Val Leu Ala Trp Gly Phe Leu  
 1 5 10 15



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<210> 75
<211> 56
<212> PRT
<213> Homo sapiens
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<210> 76
<211> 59
<212> PRT
<213> Homo sapiens
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<400> 76  
Met Thr Pro Ser Leu Leu Ser Glu Lys Leu Cys Ser Leu Phe Phe Val  
1 5 10 15







130					135					140					
Ser	Tyr	Ile	Trp	Val	Ile	Lys	Cys	Ser	Arg	Met	His	Leu	His	Xaa	Phe
145					150					155					160
Ser	Cys	Tyr	Asp	Asn	Phe	Val	Lys	Ser	Asn	Met	Phe	Gly	Ala	Pro	Glu
				165					170					175	
His	Asn	Thr	Asp	Phe	Gly	Ala	Phe	Lys	His	Leu	Tyr	Met	Leu	Leu	Val
			180					185					190		
Asn	Ala	Phe	Asn	Leu	Ser	Gln	Asn	Ser	Leu	Ser	Lys	Lys	Ser	Leu	Asn
		195					200					205			
Val	Trp	Asn	Lys	Asp	Ser	Ile	Ala	Ser	Asn	Cys	Arg	Ser	Ser	Pro	Ser
	210					215					220				
His	Thr	Thr	Asn	Gly	Cys	Gln	Gly	Glu	Lys	Val	Arg	Thr	Cys	Glu	Lys
225				230						235					240
Ser	Asp	Glu	Ser	Ala	Met	Ser	Phe	Tyr	Pro	Pro	Ser	Leu	Asn	Asp	Ala
				245					250					255	
Ser	Phe	Thr	Leu	Ile	Gly	Phe	Ser	Lys	Gly	Cys	Val	Xaa	Leu	Asn	Gln
			260					265					270		
Leu	Leu	Phe	Glu	Leu	Lys	Glu	Ala	Lys	Lys	Asp	Lys	Asn	Ile	Asp	Ala
		275					280					285			
Phe	Ile	Lys	Ser	Ile	Arg	Thr	Met	Tyr	Trp	Leu	Asp	Gly	Gly	His	Ser
	290					295					300				
Gly	Gly	Ser	Asn	Thr	Trp	Val	Thr	Tyr	Pro	Glu	Val	Leu	Lys	Glu	Phe
305				310						315					320
Ala	Gln	Thr	Gly	Ile	Ile	Val	His	Thr	His	Val	Thr	Pro	Tyr	Gln	Val
				325					330					335	
Arg	Asp	Pro	Met	Arg	Ser	Trp	Ile	Gly	Lys	Glu	Xaa	Lys	Lys	Phe	Val
			340					345					350		
Gln	Ile	Leu	Gly	Asp	Leu	Gly	Met	Gln	Val	Thr	Ser	Gln	Ile	His	Phe
		355					360					365			
Thr	Lys	Glu	Ala	Pro	Ser	Ile	Glu	Asn	His	Phe	Arg	Val	His	Glu	Val
	370					375					380				

Phe  
385

<210> 78  
 <211> 292  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (288)  
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>  
 <221> SITE  
 <222> (289)  
 <223> Xaa equals any of the naturally occurring L-amino acids







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<210> 80
<211> 1010
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (25)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (104)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (194)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (362)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (525)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (643)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (649)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (656)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (660)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>

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Ala	Arg	Leu	Ile	Lys	Lys	Lys	Arg	Glu	Lys	Asn	Gln	Ile	Asp	Ala	Ile
		115					120					125			
Lys	Asn	Asp	Lys	Gly	Asp	Ile	Thr	Thr	Asp	Pro	Thr	Glu	Ile	Gln	Thr
	130					135					140				
Thr	Ile	Arg	Glu	Tyr	Tyr	Lys	His	Leu	Tyr	Ala	Asn	Lys	Leu	Glu	Asn
145					150					155					160
Leu	Glu	Glu	Met	Asp	Lys	Phe	Leu	Asp	Thr	Tyr	Thr	Leu	Pro	Arg	Leu
				165					170					175	
Asn	Gln	Glu	Glu	Val	Glu	Ser	Leu	Asn	Arg	Pro	Ile	Thr	Gly	Ser	Glu
			180					185					190		
Ile	Xaa	Ala	Ile	Ile	Asn	Ser	Leu	Pro	Thr	Lys	Lys	Ser	Pro	Gly	Pro
		195					200					205			
Asp	Gly	Phe	Thr	Ala	Glu	Phe	Tyr	Gln	Arg	Tyr	Lys	Glu	Glu	Leu	Val
	210					215					220				
Pro	Phe	Leu	Leu	Lys	Leu	Phe	Gln	Ser	Ile	Glu	Lys	Glu	Gly	Ile	Leu
225					230					235					240
Pro	Asn	Ser	Phe	Tyr	Glu	Ala	Ser	Ile	Ile	Leu	Ile	Pro	Lys	Pro	Gly
				245					250					255	
Arg	Asp	Thr	Thr	Lys	Lys	Glu	Asn	Phe	Arg	Pro	Ile	Ser	Leu	Met	Asn
			260					265					270		
Ile	Asp	Ala	Lys	Ile	Leu	Asn	Lys	Ile	Leu	Ala	Asn	Arg	Ile	Gln	Gln
		275					280					285			
His	Ile	Lys	Lys	Leu	Ile	His	His	Asp	Gln	Val	Gly	Phe	Ile	Pro	Gly
	290					295					300				
Met	Gln	Gly	Trp	Phe	Asn	Ile	Arg	Lys	Ser	Ile	Asn	Val	Ile	Gln	His
305					310					315					320
Ile	Asn	Arg	Thr	Lys	Asp	Lys	Asn	His	Met	Ile	Ile	Ser	Ile	Asp	Ala
				325					330					335	
Glu	Lys	Ala	Phe	Asp	Lys	Ile	Gln	Gln	Pro	Phe	Met	Leu	Lys	Thr	Leu
			340					345					350		
Asn	Lys	Leu	Gly	Ile	Asp	Gly	Thr	Tyr	Xaa	Lys	Ile	Ile	Arg	Ala	Ile
		355					360					365			
Tyr	Asp	Lys	Pro	Thr	Ala	Asn	Ile	Ile	Leu	Asn	Gly	Gln	Lys	Leu	Glu
	370					375					380				
Ala	Phe	Pro	Leu	Lys	Thr	Gly	Thr	Arg	Gln	Gly	Cys	Pro	Leu	Ser	Pro
385					390					395					400
Leu	Leu	Phe	Asn	Ile	Val	Leu	Glu	Val	Leu	Ala	Arg	Ala	Ile	Arg	Gln
				405					410					415	
Glu	Lys	Glu	Ile	Lys	Gly	Ile	Gln	Leu	Gly	Lys	Glu	Glu	Val	Lys	Leu
			420					425					430		
Ser	Leu	Phe	Ala	Asp	Asp	Met	Ile	Val	Tyr	Leu	Glu	Asn	Pro	Ile	Val
		435					440					445			
Ser	Ala	Gln	Asn	Leu	Leu	Lys	Leu	Ile	Ser	Asn	Phe	Ser	Lys	Val	Ser
	450					455					460				



Gly 465	Tyr	Lys	Ile	Asn	Val 470	Gln	Lys	Ser	Gln	Ala 475	Phe	Leu	Tyr	Thr	Asn 480
Asn	Arg	Gln	Thr	Glu 485	Ser	Gln	Ile	Met	Ser 490	Glu	Leu	Pro	Phe	Thr 495	Ile
Ala	Ser	Lys	Arg 500	Ile	Lys	Tyr	Leu	Gly 505	Ile	Gln	Leu	Thr	Arg 510	Asp	Val
Lys	Asp	Leu 515	Phe	Lys	Glu	Asn	Tyr 520	Lys	Pro	Leu	Leu	Xaa 525	Glu	Ile	Lys
Glu	Asp 530	Thr	Asn	Lys	Trp	Lys 535	Asn	Ile	Pro	Cys	Ser 540	Trp	Val	Gly	Arg
Ile 545	Asn	Ile	Val	Lys	Met 550	Ala	Ile	Leu	Pro	Lys 555	Val	Ile	Tyr	Arg	Phe 560
Asn	Ala	Ile	Pro	Ile 565	Lys	Leu	Pro	Met	Thr 570	Phe	Phe	Thr	Glu	Leu 575	Glu
Lys	Thr	Thr	Leu 580	Lys	Phe	Ile	Trp	Asn 585	Gln	Lys	Arg	Ala	Arg 590	Ile	Ala
Lys	Ser	Ile 595	Leu	Ser	Gln	Lys	Asn 600	Lys	Ala	Gly	Gly	Ile 605	Thr	Leu	Pro
Asp	Phe 610	Lys	Leu	Tyr	Tyr	Lys 615	Ala	Thr	Val	Thr	Lys 620	Thr	Ala	Trp	Tyr
Trp 625	Tyr	Gln	Asn	Arg	Asp 630	Ile	Asp	Gln	Trp	Asn 635	Arg	Thr	Glu	Pro	Ser 640
Glu	Ile	Xaa	Pro	His 645	Ile	Tyr	Asn	Xaa	Leu 650	Ile	Phe	Asp	Lys	Pro 655	Xaa
Lys	Asn	Lys	Xaa 660	Trp	Gly	Lys	Asp	Ser 665	Leu	Phe	Asn	Lys	Trp 670	Cys	Trp
Glu	Asn	Trp 675	Leu	Ala	Ile	Cys	Arg 680	Lys	Leu	Lys	Leu	Asp 685	Pro	Phe	Leu
Thr	Pro 690	Tyr	Thr	Lys	Ile	Asn 695	Ser	Arg	Trp	Ile	Lys 700	Asp	Leu	Asn	Val
Arg 705	Pro	Lys	Thr	Ile	Lys 710	Thr	Leu	Glu	Glu	Asn 715	Leu	Gly	Asn	Thr	Ile 720
Gln	Asp	Ile	Gly	Met 725	Gly	Lys	Asp	Phe	Met 730	Xaa	Lys	Thr	Pro	Lys 735	Ala
Met	Ala	Thr	Lys 740	Ala	Lys	Ile	Asp	Lys 745	Trp	Asp	Leu	Ile	Lys 750	Leu	Lys
Ser	Phe 755	Cys	Thr	Ala	Lys	Glu	Thr 760	Thr	Ile	Arg	Val	Asn 765	Arg	Gln	Pro
Thr	Xaa 770	Trp	Glu	Lys	Ile	Phe 775	Ala	Xaa	Tyr	Ser	Ser 780	Asp	Lys	Gly	Leu
Ile 785	Ser	Arg	Ile	Tyr	Xaa 790	Glu	Leu	Lys	Gln	Ile 795	Tyr	Lys	Lys	Lys	Xaa 800
Asn	Asn	Pro	Ile 805	Lys	Lys	Trp	Ala	Lys	Asp 810	Met	Asn	Arg	His 815	Phe	Ser



Lys	Glu	Asp	Ile	Tyr	Ala	Ala	Lys	Xaa	His	Met	Lys	Lys	Cys	Ser	Ser		
			820				825							830			
Ser	Leu	Ala	Ile	Arg	Glu	Met	Gln	Ile	Lys	Thr	Thr	Met	Arg	Tyr	His		
			835				840							845			
Leu	Thr	Pro	Val	Arg	Met	Ala	Ile	Ile	Lys	Lys	Ser	Gly	Asn	Asn	Arg		
			850				855							860			
Cys	Trp	Arg	Gly	Cys	Gly	Glu	Ile	Gly	Thr	Leu	Leu	His	Cys	Trp	Trp		
			865				870							875			
Asp	Cys	Lys	Leu	Val	Gln	Pro	Leu	Trp	Lys	Ser	Val	Trp	Arg	Phe	Leu		
			885				890							895			
Arg	Asp	Leu	Glu	Leu	Glu	Ile	Pro	Phe	Asp	Pro	Ala	Ile	Pro	Leu	Leu		
			900				905							910			
Gly	Ile	Tyr	Pro	Lys	Asp	Tyr	Lys	Ser	Cys	Cys	Tyr	Lys	Asp	Thr	Cys		
			915				920							925			
Thr	Arg	Met	Phe	Ile	Ala	Ala	Leu	Phe	Thr	Ile	Ala	Lys	Thr	Trp	Asn		
			930				935							940			
Gln	Pro	Lys	Cys	Pro	Thr	Met	Ile	Asp	Trp	Ile	Lys	Lys	Met	Trp	His		
			945				950							955			
Ile	Tyr	Thr	Met	Glu	Tyr	Tyr	Ala	Ala	Ile	Lys	Asn	Asp	Glu	Phe	Met		
			965				970							975			
Ser	Phe	Val	Gly	Thr	Trp	Met	Lys	Leu	Glu	Xaa	Ile	Ile	Leu	Ser	Lys		
			980				985							990			
Leu	Ser	Gln	Xaa	Gln	Lys	Thr	Lys	His	Arg	Xaa	Phe	Ser	Leu	Ile	Gly		
			995				1000							1005			
Gly	Asn																
			1010														
<210> 81																	
<211> 120																	
<212> PRT																	
<213> Homo sapiens																	
<400> 81																	
Met	Arg	Leu	Thr	Arg	Lys	Arg	Leu	Cys	Ser	Phe	Leu	Ile	Ala	Leu	Tyr		
			1				5				10				15		
Cys	Leu	Phe	Ser	Leu	Tyr	Ala	Ala	Tyr	His	Val	Phe	Phe	Gly	Arg	Arg		
			20				25							30			
Arg	Gln	Ala	Pro	Ala	Gly	Ser	Pro	Arg	Gly	Leu	Arg	Lys	Gly	Ala	Ala		
			35				40							45			
Pro	Ala	Arg	Glu	Arg	Arg	Gly	Arg	Glu	Gln	Ser	Thr	Leu	Glu	Ser	Glu		
			50				55							60			
Glu	Trp	Asn	Pro	Trp	Glu	Gly	Asp	Glu	Lys	Asn	Glu	Gln	Gln	His	Arg		
			65				70							75			
Phe	Lys	Thr	Ser	Leu	Gln	Ile	Leu	Asp	Lys	Ser	Thr	Lys	Gly	Lys	Thr		
			85				90							95			
Asp	Leu	Ser	Val	Gln	Ile	Trp	Gly	Lys	Ala	Ala	Ile	Val	Gln	Ala	Gly		
			100				105							110			











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<210> 87
<211> 90
<212> PRT
<213> Homo sapiens
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<400> 87  
Met Ala Val Thr Trp Xaa Gln Ala Leu Leu Arg Ala Leu Cys Ile Ser  
1 5 10 15

Ala His Pro Ser Met Arg Trp Arg Gly Arg Pro Leu Ala Arg Thr Leu  
35 40 45

Gly Ala Leu Arg Ala Gly Arg Thr Pro Val Pro Ser Ser Glu Glu Ala  
65 70 75 80

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<210> 88
<211> 25
<212> PRT
<213> Homo sapiens
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<400> 88  
Met Gln Ile Leu Leu Leu Phe Tyr Phe Ser Arg Phe Leu Ala Pro Ser  
1 5 10 15

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<210> 89
<211> 50
<212> PRT
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<400> 89

Pro	Arg
	50

<213> Homo sapiens

His Thr Thr Asn Gly Cys Gln Gly Glu Lys Val Arg Thr Cys Glu Lys  
225 230 235 240







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<210> 93
<211> 43
<212> PRT
<213> Homo sapiens
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<210> 94
<211> 44
<212> PRT
<213> Homo sapiens
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<210> 95
<211> 37
<212> PRT
<213> Homo sapiens
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<210> 96
<211> 43
<212> PRT
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<210> 100
<211> 40
<212> PRT
<213> Homo sapiens
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<210> 101
<211> 41
<212> PRT
<213> Homo sapiens
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&lt;400&gt; 101

Lys Met Leu Lys Arg Leu Lys Thr Gln Met Ala Glu Val Arg Cys Met  
 1 5 10 15

Lys Thr Asp Val Lys Asn Thr Leu Ser Glu Ile Lys Ser Ser Ser Ala  
 20 25 30

Ala Ser Gly Asp Met Gln Thr Ser Leu  
 35 40

&lt;210&gt; 102

&lt;211&gt; 41

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 102

Phe Ser Ala Asp Gln Ala Ala Leu Ala Ala Cys Gly Thr Glu Asn Ser  
 1 5 10 15

Gly Arg Leu Gln Asp Leu Gly Met Glu Leu Leu Ala Lys Ser Ser Val  
 20 25 30

Ala Asn Cys Tyr Ile Arg Asn Ser Thr  
 35 40

&lt;210&gt; 103

&lt;211&gt; 42

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 103

Asn Lys Lys Ser Asn Ser Pro Lys Pro Ala Arg Ser Ser Val Ala Gly  
 1 5 10 15

Ser Leu Ser Leu Arg Arg Ala Val Asp Pro Gly Glu Asn Ser Arg Ser  
 20 25 30

Lys Gly Asp Cys Gln Thr Leu Ser Glu Gly  
 35 40

&lt;210&gt; 104

&lt;211&gt; 44

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 104

Ser Pro Gly Ser Ser Gln Ser Gly Ser Arg His Ser Ser Pro Arg Ala  
 1 5 10 15

Leu Ile His Gly Ser Ile Gly Asp Ile Leu Pro Lys Thr Glu Asp Arg  
 20 25 30

Gln Cys Lys Ala Leu Asp Ser Asp Ala Val Val Val  
 35 40

&lt;210&gt; 105

&lt;211&gt; 42

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 105



<400> 109  
His Ala Ser Gly Trp Ala Cys Leu Gly Arg Arg Arg Cys Arg Gly Phe  
1 5 10 15



Ser	Phe	Arg	Pro 20	Leu	His	Gly	Gly	Gly 25	Cys	Leu	Thr	Gly	Ser 30	Pro	Ser
Gly	Met	Arg 35	Leu	Thr	Arg	Lys	Arg 40	Leu	Cys	Ser	Phe	Leu 45	Ile	Ala	Leu
Tyr	Cys 50	Leu	Phe	Ser	Leu	Tyr 55	Ala	Ala	Tyr	His	Val 60	Phe	Phe	Gly	Arg
Arg 65	Arg	Gln	Ala	Pro	Ala 70	Gly	Ser	Pro	Arg	Gly 75	Leu	Arg	Lys	Gly	Ala 80
Ala	Pro	Ala	Arg	Glu 85	Arg	Arg	Gly	Arg	Glu 90	Gln	Ser	Thr	Leu	Glu 95	Ser
Glu	Glu	Trp	Asn 100	Pro	Trp	Glu	Gly	Asp 105	Glu	Lys	Asn	Glu	Gln 110	Gln	His
Arg	Phe	Lys 115	Thr	Ser	Leu	Gln	Ile 120	Leu	Asp	Lys	Ser	Thr 125	Lys	Gly	Lys
Thr	Asp 130	Leu	Ser	Val	Gln	Ile 135	Trp	Gly	Lys	Ala	Ala 140	Ile	Gly	Leu	Tyr
Leu 145	Trp	Glu	His	Ile	Phe 150	Glu	Gly	Leu	Leu	Asp 155	Pro	Ser	Asp	Val	Thr 160
Ala	Gln	Trp	Arg	Glu 165	Gly	Lys	Ser	Ile	Val 170	Gly	Arg	Thr	Gln	Tyr 175	Ser
Phe	Ile	Thr	Gly 180	Pro	Ala	Val	Ile	Pro 185	Gly	Tyr	Phe	Ser	Val 190	Asp	Val
Asn	Asn	Val 195	Val	Leu	Ile	Leu	Asn 200	Gly	Arg	Glu	Lys	Ala 205	Lys	Ile	Phe
Tyr	Ala 210	Thr	Gln	Trp	Leu	Leu 215	Tyr	Ala	Gln	Asn	Leu 220	Val	Gln	Ile	Gln
Lys 225	Leu	Gln	His	Leu	Ala 230	Val	Val	Leu	Leu	Gly 235	Asn	Glu	His	Cys	Asp 240
Asn	Glu	Trp	Ile	Asn 245	Pro	Phe	Leu	Lys	Arg 250	Asn	Gly	Gly	Phe	Val 255	Glu
Leu	Leu	Phe	Ile 260	Ile	Tyr	Asp	Ser	Pro 265	Trp	Ile	Asn	Asp	Val 270	Asp	Val
Phe	Gln	Trp 275	Pro	Leu	Gly	Val	Ala 280	Thr	Tyr	Arg	Asn	Phe 285	Pro	Val	Val
Glu	Ala 290	Ser	Trp	Ser	Met	Leu 295	His	Asp	Glu	Arg	Pro 300	Tyr	Leu	Cys	Asn
Phe 305	Leu	Gly	Thr	Ile	Tyr 310	Glu	Asn	Ser	Ser	Arg 315	Gln	Ala	Leu	Met	Asn 320
Ile	Leu	Lys	Lys	Asp 325	Gly	Asn	Asp	Lys	Leu 330	Cys	Trp	Val	Ser	Ala 335	Arg
Glu	His	Trp	Gln 340	Pro	Gln	Glu	Thr	Asn 345	Glu	Ser	Leu	Lys	Asn 350	Tyr	Gln
Asp	Ala	Leu 355	Leu	Gln	Ser	Asp	Leu 360	Thr	Leu	Cys	Pro	Val 365	Gly	Val	Asn







Glu Ala Arg Gly Gly  
85

<210> 112  
<211> 72  
<212> PRT  
<213> Homo sapiens

<400> 112  
Tyr Arg His Leu Trp Arg Asp Arg Val Cys Gln Leu Cys His Pro Ser  
1 5 10 15  
Pro Val His Ala Ala Ala His Leu Ser Leu Glu Thr Leu Thr Ala Phe  
20 25 30  
Val Leu Pro Phe Gly Leu Met Leu Gly Cys Tyr Ser Val Thr Leu Ala  
35 40 45  
Arg Leu Arg Gly Ala Arg Trp Gly Ser Gly Arg His Gly Ala Arg Val  
50 55 60  
Gly Arg Leu Val Ser Ala Ile Val  
65 70

<210> 113  
<211> 172  
<212> PRT  
<213> Homo sapiens

<400> 113  
Ala Pro Arg Leu Leu Leu Leu Asn Leu Ser Ala Ser Pro Gly Pro Gln  
1 5 10 15  
Ser Cys Leu His Pro Ala Trp Glu Arg Asp Thr Ala Glu Leu Glu Asp  
20 25 30  
Phe Ala Gly His Arg His Ser Leu Pro Ala Ala Gly Gly Ala Ala Gly  
35 40 45  
Ala Ala Trp Gln Arg Leu Arg Gly Val Glu Leu Gly Gly Leu Ala Ala  
50 55 60  
Cys Thr Gly Ala Thr Ala Gly Gly His Ala Cys Ala Ala Pro Gly Ala  
65 70 75 80  
Gly Arg Arg Arg Gly Ala Ala Ala His Ala Ala Leu Cys Gly Leu Pro  
85 90 95  
Asp Pro Ala Ser Leu Ala Ala Gly Pro Gly Gly Leu Gln Gly Gly Val  
100 105 110  
Leu Arg Val Arg Ala Gln His Val Arg Gln Arg Ala Ala His Arg Pro  
115 120 125  
Ala Gln Pro Ala Ala Leu Pro Arg Gly His Pro Pro Leu Pro Gly Ala  
130 135 140  
Ser Val Arg Ser Pro Ala Leu Ala Arg Arg Leu Leu Leu Ala Val Trp  
145 150 155 160  
Leu Ala Ala Leu Leu Leu Ala Val Pro Ala Ala Val  
165 170



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<210> 116
<211> 77
<212> PRT
<213> Homo sapiens
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$\langle 220 \rangle$ 

<222> (39)

<400> 118

Val Arg Pro Ser Pro Pro Ser Ala Ser Arg Leu Arg Arg Leu Pro Pro  
20 25 30

<210> 119

 $\langle 211 \rangle$  44

<212> PRT

<400> 119

Cys Gly Ser Lys Met Ser Asp Ile Gly Asp Trp Phe Arg Ser Ile Pro  
20 25 30

 $\langle 210 \rangle$  120

$\langle 211 \rangle$	45
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<212> PRT

<400> 120

Leu Trp Pro Glu Ala Phe Leu Tyr Arg Phe Gln Ile Trp Arg Pro Ile  
20 25 30

 $\langle 210 \rangle$  121

$\langle 211 \rangle$  31

<212> PRT

<400> 121

Leu Glu Thr Gly Ala Phe Asp Gly Arg Pro Ala Asp Tyr Leu Phe  
20 25 30

<210> 122

<211> 314

<212> PRT



$\langle 220 \rangle$ 

<222> (39)

 $\langle 220 \rangle$  $\langle 222 \rangle$  (300)

<400> 122

Val Arg Pro Ser Pro Pro Ser Ala Ser Arg Leu Arg Arg Leu Pro Pro  
20 25 30

Arg His Leu Thr Val Ala Xaa Arg Pro Arg Arg Glu Gly Val Gly Thr  
35 40 45

Gly Ser Arg Ala Val Leu Cys Ile Leu Ala Thr Cys Gly Ser Lys Met  
50 55 60

Ser Asp Ile Gly Asp Trp Phe Arg Ser Ile Pro Ala Ile Thr Arg Tyr  
65 70 75 80

Trp Phe Ala Ala Thr Val Ala Val Pro Leu Val Gly Lys Leu Gly Leu  
85 90 95

Ile Ser Pro Ala Tyr Leu Phe Leu Trp Pro Glu Ala Phe Leu Tyr Arg  
100 105 110

Phe Gln Ile Trp Arg Pro Ile Thr Ala Thr Phe Tyr Phe Pro Val Gly  
115 120 125

Pro Gly Thr Gly Phe Leu Tyr Leu Val Asn Leu Tyr Phe Leu Tyr Gln  
130 135 140

Tyr Ser Thr Arg Leu Glu Thr Gly Ala Phe Asp Gly Arg Pro Ala Asp  
145 150 155 160

Tyr Leu Phe Met Leu Leu Phe Asn Trp Ile Cys Ile Val Ile Thr Gly  
165 170 175

Leu Ala Met Asp Met Gln Leu Leu Met Ile Pro Leu Ile Met Ser Val  
180 185 190

Leu Tyr Val Trp Ala Gln Leu Asn Arg Asp Met Ile Val Ser Phe Trp  
195 200 205

Phe Gly Thr Arg Phe Lys Ala Cys Tyr Leu Pro Trp Val Ile Leu Gly  
210 215 220

Phe Asn Tyr Ile Ile Gly Gly Ser Val Ile Asn Glu Leu Ile Gly Asn  
225 230 235 240

Leu Val Gly His    Leu Tyr Phe Phe Leu Met Phe Arg Tyr Pro Met Asp  
                    245                         250                         255

Leu Gly Gly Arg Asn Phe Leu Ser Thr Pro Gln Phe Leu Tyr Arg Trp  
260 265 270

Leu Pro Ser Arg Arg Gly Gly Val Ser Gly Phe Gly Val Pro Pro Ala  
275 280 285



Ser Met Arg Arg Ala Ala Asp Gln Asn Gly Gly Xaa Gly Arg His Asn  
 290 295 300

Trp Gly Gln Gly Phe Arg Leu Gly Asp Gln  
 305 310

<210> 123  
 <211> 172  
 <212> PRT  
 <213> Homo sapiens

<400> 123  
 Ala Ala Arg Gly Leu Tyr Asp Tyr Gly Ser Gly Leu Cys Trp Ala Trp  
 1 5 10 15  
 Ala Ala Arg Pro Ser Ser Phe Val Ser Gly Ser Ser Arg Glu Ala Pro  
 20 25 30  
 Ser Ala Thr Ala Ala Pro Ser Trp Thr Arg Ser Val Thr Ala Ala Ser  
 35 40 45  
 Ala Ala Ala Ala Ser Arg Met Ala Met Cys Ser Ser Thr Arg Pro Ala  
 50 55 60  
 Arg Leu Leu Leu Pro Pro Pro Thr Thr Pro Ser Pro Arg Pro Arg Thr  
 65 70 75 80  
 Leu Thr Pro Val Asp Pro Cys Ser Gly Gly Cys Arg Leu Thr Ser Lys  
 85 90 95  
 Asp His Thr Pro Arg Val Gly Thr Gly Gln Gly Arg Gly Gln Gly Thr  
 100 105 110  
 Phe Trp Leu Ser Arg Asp Glu Gly Tyr Phe Ala Glu Asp Thr Arg Ile  
 115 120 125  
 Gly His Phe Gln Asp Ser Leu Pro Ala Pro Leu Pro Leu Pro Ser Phe  
 130 135 140  
 Glu Ala Leu Ile Lys His Lys Ser Gly Ser Pro Gly Ala Val Cys Gln  
 145 150 155 160  
 Arg Trp Ala Gly Gly Glu Thr Asp Arg Gly Cys Gly  
 165 170

<210> 124  
 <211> 39  
 <212> PRT  
 <213> Homo sapiens

<400> 124  
 Ala Ala Arg Gly Leu Tyr Asp Tyr Gly Ser Gly Leu Cys Trp Ala Trp  
 1 5 10 15  
 Ala Ala Arg Pro Ser Ser Phe Val Ser Gly Ser Ser Arg Glu Ala Pro  
 20 25 30  
 Ser Ala Thr Ala Ala Pro Ser  
 35

<210> 125  
 <211> 39







```
<210> 130
<211> 103
<212> PRT
<213> Homo sapiens
```

```
<220>
<221> SITE
<222> (42)
<223> Xaa equals any of the naturally occurring L-amino acids
```

```

<400> 130
Met Phe Leu Leu Asp Gly Ser Asn Trp Ile Leu His Cys Pro Ile Thr
  1           5           10           15
Leu Arg Thr Tyr Thr Thr Asn Leu Ser Ile Lys Phe Ser Lys Cys Ser
          20           25
Val Asn Ile Tyr Ser Leu Glu Asn Lys Xaa Phe Phe Ser Lys Lys Lys
      35           40           45
Lys Lys Lys Arg Lys Glu Asn Asn Pro Gly Asn Lys Ile Ser Asn Gly
    50           55           60
Glu Ile Ser Val Thr Leu Thr Gly Ile Cys Lys Ile Phe Trp Lys Arg
  65           70           75           80
Ala Pro Phe Phe Phe His Phe Gln Ser Tyr Leu Trp Cys Ser Tyr Arg
          85           90           95
Val Gln Thr Ser Arg Ser Phe
          100

```

```
<210> 131
<211> 211
<212> PRT
<213> Homo sapiens
```

<400> 131																
Gly	Arg	Gly	Pro	Thr	Ala	Pro	Ala	Val	Arg	Asp	Pro	Asn	Ala	Ile	Pro	
1				5					10					15		
Ala	Gln	Arg	Ser	Met	Ala	Ala	Thr	Asp	Ser	Met	Arg	Gly	Glu	Ala	Pro	
			20					25					30			
Gly	Ala	Glu	Thr	Pro	Ser	Leu	Arg	His	Arg	Gly	Gln	Ala	Ala	Gln	Pro	
		35					40					45				
Glu	Pro	Ser	Thr	Gly	Phe	Thr	Ala	Thr	Pro	Pro	Ala	Pro	Asp	Ser	Pro	
	50					55					60					
Gln	Glu	Pro	Leu	Val	Leu	Arg	Leu	Lys	Phe	Leu	Asn	Asp	Ser	Glu	Gln	
65					70					75					80	
Val	Ala	Arg	Ala	Trp	Pro	His	Asp	Thr	Ile	Gly	Ser	Leu	Lys	Arg	Thr	
				85					90					95		
Gln	Phe	Pro	Gly	Arg	Glu	Gln	Gln	Val	Arg	Leu	Ile	Tyr	Gln	Gly	Gln	
			100					105					110			
Leu	Leu	Gly	Asp	Asp	Thr	Gln	Thr	Leu	Gly	Ser	Leu	His	Leu	Pro	Pro	
		115					120					125				















```
<210> 139
<211> 53
<212> PRT
<213> Homo sapiens
```

```
<210> 140
<211> 53
<212> PRT
<213> Homo sapiens

<400> 140
```



```
<210> 141
<211> 53
<212> PRT
<213> Homo sapiens
```

```
<210> 142
<211> 53
<212> PRT
<213> Homo sapiens
```

```
<210> 143
<211> 54
<212> PRT
<213> Homo sapiens
```

<400> 143  
Ala Glu Gly Ser Leu Tyr Ile Trp Ser Val Leu Thr Gly Lys Val Glu  
1 5 10 15  
Lys Val Leu Ser Lys Gln His Ser Ser Ser Ile Asn Ala Val Ala Trp  
20 25 30  
Ser Pro Ser Gly Ser His Val Val Ser Val Asp Lys Gly Cys Lys Ala  
35 40 45



Gly Phe Ile Leu Asn Leu Gly Ala Arg Leu Ile Val Gln Pro Gln Ala



```
<210> 147
<211> 40
<212> PRT
<213> Homo sapiens
```

```
<210> 148
<211> 40
<212> PRT
<213> Homo sapiens
```

```

<400> 148
Arg Leu Ile Val Gln Pro Gln Ala Ala Leu Ala Ser Arg Gly Leu Arg
  1                               5                10                15

Gly Gln Gly Leu Pro Cys Glu Thr Gln Val Xaa Lys Arg Thr Leu Arg
                20                25                30

Pro Gly Ala Val Gly Trp Leu Val
      35                40

```

<400> 149  
His Lys Gly Arg Arg Ala Leu Ser Ile Ser Arg Lys Ser Ala Leu Val  
1 5 10 15  
Ser Leu Gly Val Met Tyr Val Gly Pro Gly Lys Arg Pro Gly Val Val  
20 25 30



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<210> 150
<211> 60
<212> PRT
<213> Homo sapiens
```

```

<400> 150
His Ile Ile Phe Phe Arg Lys Trp Ser Thr Leu Ala Phe Ile Ile Pro
  1             5             10             15

Tyr Ser Ser Val Ser Gly Ile Ile Ser Ile Ala Ser Phe Met Ser Val
      20             25             30

Ala Ser Glu Ile Ala Ser Leu Val Phe Leu Arg Lys Asn Thr Thr Phe
      35             40             45

Trp Ser Arg Asn Ser Ser Gly Arg Gly Val Gln Ser
      50             55             60

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```
<210> 151
<211> 110
<212> PRT
<213> Homo sapiens
```

```
<220>
<221> SITE
<222> (73)
<223> Xaa equals any of the naturally occurring L-amino acids
```

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<400> 151
Val Leu Cys Gly Pro Gly Ala Ala Thr Arg Lys Gly Ser Gln Leu Asn
  1          5          10          15
Pro Ala Val Ala Ser Pro Ala Phe Pro His Pro Gly Phe Phe Ser Leu
          20          25          30
Ser Asn Leu Gly Ser Ser Tyr Ser Ser Ser Asn Thr Met Tyr Ser Cys
          35          40          45
Pro Ser Glu Pro Leu His Arg Leu Ser Pro Leu Pro Lys Glu Thr Pro
          50          55          60
Leu Leu Ser Ser Pro Ser Pro Thr Xaa Pro Ser Gln Pro Ala Glu Leu
  65          70          75          80
Trp Phe Ile Phe Cys Ile Arg Val Lys Gly His Leu Pro Cys Gln Ser
          85          90          95
Thr Pro Thr Leu Pro Leu Gln Ser Ser Glu Met Ser Ser Leu
          100          105          110

```

```
<210> 152
<211> 39
<212> PRT
<213> Homo sapiens
```

<400> 152  
Val Leu Cys Gly Pro Gly Ala Ala Thr Arg Lys Gly Ser Gln Leu Asn  
1 5 10 15



Pro Ala Val Ala Ser Pro Ala Phe Pro His Pro Gly Phe Phe Ser Leu  
                   20                  25                  30

Ser Asn Leu Gly Ser Ser Tyr  
                   35

<210> 153  
 <211> 40  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (34)  
 <223> Xaa equals any of the naturally occurring L-amino acids

<400> 153  
 Ser Ser Ser Asn Thr Met Tyr Ser Cys Pro Ser Glu Pro Leu His Arg  
       1                  5                  10                  15

Leu Ser Pro Leu Pro Lys Glu Thr Pro Leu Leu Ser Ser Pro Ser Pro  
                   20                  25                  30

Thr Xaa Pro Ser Gln Pro Ala Glu  
                   35                  40

<210> 154  
 <211> 31  
 <212> PRT  
 <213> Homo sapiens

<400> 154  
 Leu Trp Phe Ile Phe Cys Ile Arg Val Lys Gly His Leu Pro Cys Gln  
       1                  5                  10                  15

Ser Thr Pro Thr Leu Pro Leu Gln Ser Ser Glu Met Ser Ser Leu  
                   20                  25                  30

<210> 155  
 <211> 47  
 <212> PRT  
 <213> Homo sapiens

<400> 155  
 Thr Ser Ser Pro Gln Arg Arg Leu Pro Ala Gly Pro Arg Pro Pro Thr  
       1                  5                  10                  15

Val Glu Pro Pro Ala Glu Pro Pro Ala Glu Val Pro Pro Ser Gly Thr  
                   20                  25                  30

Pro Pro Pro Pro Ser Thr Ser Glu Pro Leu Ser Arg Arg Arg Pro  
                   35                  40                  45

<210> 156  
 <211> 432  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (111)  
 <223> Xaa equals any of the naturally occurring L-amino acids



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<220>
<221> SITE
<222> (115)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (206)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (316)
<223> Xaa equals any of the naturally occurring L-amino acids

<220>
<221> SITE
<222> (395)
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 156
Thr Ser Ser Pro Gln Arg Arg Leu Pro Ala Gly Pro Arg Pro Pro Thr
  1           5           10           15

Val Glu Pro Pro Ala Glu Pro Pro Ala Glu Val Pro Pro Ser Gly Thr
      20           25           30

Pro Pro Pro Pro Ser Thr Ser Glu Pro Leu Ser Arg Arg Arg Pro Met
  35           40           45

Trp Gly Phe Arg Leu Leu Arg Ser Pro Pro Leu Leu Leu Leu Pro
  50           55           60

Gln Leu Gly Ile Gly Asn Ala Ser Ser Cys Ser Gln Ala Arg Thr Met
  65           70           75           80

Asn Pro Gly Gly Ser Gly Gly Ala Arg Cys Ser Leu Ser Ala Glu Val
      85           90           95

Arg Arg Arg Gln Cys Leu Gln Leu Ser Thr Val Pro Gly Ala Xaa Pro
      100           105           110

Gln Arg Xaa Asn Glu Leu Leu Leu Leu Ala Ala Ala Gly Glu Gly Leu
      115           120           125

Glu Arg Gln Asp Leu Pro Gly Asp Pro Ala Lys Glu Glu Pro Gln Pro
      130           135           140

Pro Pro Gln His His Val Leu Tyr Phe Pro Gly Asp Val Gln Asn Tyr
      145           150           155           160

His Glu Ile Met Thr Arg His Pro Glu Asn Tyr Gln Trp Glu Asn Trp
      165           170           175

Ser Leu Glu Asn Val Ala Thr Ile Leu Ala His Arg Phe Pro Asn Ser
      180           185           190

Tyr Ile Trp Val Ile Lys Cys Ser Arg Met His Leu His Xaa Phe Ser
      195           200           205

Cys Tyr Asp Asn Phe Val Lys Ser Asn Met Phe Gly Ala Pro Glu His
      210           215           220

Asn Thr Asp Phe Gly Ala Phe Lys His Leu Tyr Met Leu Leu Val Asn
      225           230           235           240

```



Ala	Phe	Asn	Leu	Ser 245	Gln	Asn	Ser	Leu	Ser 250	Lys	Lys	Ser	Leu	Asn 255	Val
Trp	Asn	Lys	Asp 260	Ser	Ile	Ala	Ser	Asn 265	Cys	Arg	Ser	Ser	Pro 270	Ser	His
Thr	Thr	Asn 275	Gly	Cys	Gln	Gly	Glu 280	Lys	Val	Arg	Thr	Cys 285	Glu	Lys	Ser
Asp	Glu 290	Ser	Ala	Met	Ser	Phe 295	Tyr	Pro	Pro	Ser	Leu 300	Asn	Asp	Ala	Ser
Phe 305	Thr	Leu	Ile	Gly	Phe 310	Ser	Lys	Gly	Cys	Val 315	Xaa	Leu	Asn	Gln	Leu 320
Leu	Phe	Glu	Leu	Lys 325	Glu	Ala	Lys	Lys	Asp 330	Lys	Asn	Ile	Asp	Ala 335	Phe
Ile	Lys	Ser	Ile 340	Arg	Thr	Met	Tyr	Trp 345	Leu	Asp	Gly	Gly	His 350	Ser	Gly
Gly	Ser	Asn 355	Thr	Trp	Val	Thr	Tyr 360	Pro	Glu	Val	Leu	Lys 365	Glu	Phe	Ala
Gln	Thr 370	Gly	Ile	Ile	Val	His 375	Thr	His	Val	Thr	Pro 380	Tyr	Gln	Val	Arg
Asp 385	Pro	Met	Arg	Ser	Trp 390	Ile	Gly	Lys	Glu	Xaa 395	Lys	Lys	Phe	Val	Gln 400
Ile	Leu	Gly	Asp	Leu 405	Gly	Met	Gln	Val	Thr 410	Ser	Gln	Ile	His	Phe 415	Thr
Lys	Glu	Ala	Pro 420	Ser	Ile	Glu	Asn	His 425	Phe	Arg	Val	His	Glu 430	Val	Phe